THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

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Letter from the Editor

During the past year the Editor and members of the Editorial Board have called upon many of their colleagues and others especially qualified to review certain manuscripts submitted to the REVIEW for publication. They are most grateful for the help given them by those whose names are listed below:

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 79 *January 1959* NUMBER 1

EXPERIMENTS ON THE ANTITUBERCULOUS ACTIVITY OF ALPHA-ETHYL-THIOISONICOTINAMIDE[1, 2]

NOËL RIST, FRANÇOISE GRUMBACH, AND DAVID LIBERMANN

(Received for publication August 1, 1958)

Introduction

The compound thioisonicotinamide (3264 Th) was synthesized by one of the present writers (D. L.) in May, 1952. This compound is not a derivative of isoniazid, but like isoniazid it is a derivative of isonicotinic acid. A major difference between thioisonicotinamide and isoniazid is that the former compound does not contain the hydrazide chain. Soon after the synthesis of the compound, it was tested for antituberculous activity, using the H37Rv strain of tubercle bacilli grown in Youmans serum medium. As the *in vitro* tests reveal that the inhibitory concentration of the thioamide was 20 γ per ml. (or approximately four hundred times less active than isoniazid), it was considered inadvisable to test the compound further in laboratory animals.

In 1954, however, Gardner, Wenis, and Lee (1) reported that thioisonicotinamide was active in experimental tuberculous infections in mice. At the same time the present writers first learned that Lee had succeeded in synthesizing this thioamide in 1951 without, however, publishing a report on the synthesis in the periodical literature (2). Accordingly, a reinvestigation of thioisonicotinamide was begun by the present writers (3). It was found that thioisonicotinamide was active both *in vitro* and *in vivo* against isoniazid-resistant as well as isoniazid-susceptible tubercle bacilli. Moreover, it was noted that thioisonicotinamide caused tubercle bacilli to lose their acid-fastness when exposed to the drug *in vitro*. As in the case of isoniazid, this loss of acid-fastness occurred only if the drug were added to an actively growing culture (4, 17). In the studies conducted with mice, it was found that thioisonicotinamide was equally active by the oral and by the subcutaneous routes, and that the degree of activity was considerably greater than had been anticipated on the basis of the *in vitro* studies. In actuality, the protective dose of the compound was found to be 50 mg. per kg., i.e., forty times greater than the protective dose of isoniazid and only one-half that of streptomycin (table 1).

Subsequent studies, beginning in 1955, were carried on with thioisonicotinamide and consisted of clinical trials in pulmonary tuberculosis in humans, clinical trials in leprosy, and a series of laboratory studies organized in an attempt to modify the structure of the thioisonicotinamide molecule.

The clinical trials in tuberculosis were carried out at the U. S. Veterans Administration Hospital at Madison, Wisconsin (5). It was found that the maximal dose of the drug that could be tolerated by the digestive tract was 1 gm. per day and it was also observed that this dosage appeared to be totally ineffective with respect to the tuberculosis. It should be noted that this dosage of 1 gm. per day is very much lower than the protective dose in the mouse (50 mg. per kg).

The clinical trials in leprosy were started because of a possible analogy with the history of diamino-diphenylsulfone (6). In association with Laviron and Beytoux, the thioisonicotinamide was administered in a total daily dosage of 1 gm. per day to patients with leprosy, and the preliminary results have been very encouraging (7).

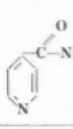
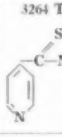
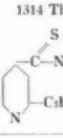
The disproportion between the relatively weak activity of thioisonicotinamide *in vitro* and its

[1] From L'Institut Pasteur, Paris, France.

[2] Presented in part at the Scientific Sessions of the International Union Against Tuberculosis, Paris, July, 1958.

TABLE 1

ANTITUBERCULOUS ACTIVITY OF THIOISONICOTINAMIDE (3264 TH) AND L-ETHYL-THIOISONICOTINAMIDE (1314 TH)

Biological Activities	Isoniazid 	Thioamides		Streptomycin
		3264 Th 	1314 Th 	
Minimal inhibiting concentration (γ per ml.)	0.05	20	0.6	1
Protective dose in mouse (mg. per kg.)	1.2	50	12	100
Toxicity in mouse LD_{50} (mg. per kg.)	200	1,000	1,000	2,000

strong activity *in vivo* suggested the possibility that the compound underwent some change in the tissues that increased its antituberculous effectiveness. Accordingly, Meltzer, Lewis, and King (8) prepared derivatives of thioisonicotinamide by methylation and oxidation of the nitrogen of the amido-groups. These derivatives were found to be of no greater activity than the original thioamide. The present writers first prepared the methyl derivative in the alpha position on the nucleus.

It should be noted that this type of chemical substitution had been carried out in the case of the isoniazid molecule by Maruyama and Inamura (9) and also by Efimovsky and Rumpf (10). These investigators had shown that the alpha-methyl derivative of isoniazid was no more active than isoniazid itself. As to the higher derivatives (the alpha-ethyl and alpha-propyl, and others), Isler and his associates have shown that their activity was very much inferior to that of isoniazid (11).

The situation was found to be quite different with respect to the derivatives of thioisonicotinamide. In this case the present writers have found that the alpha-methyl derivative was twice as effective *in vivo* and eight times as active *in vitro* as the original thioamide. Encouraged by the results of this finding, higher homologues were prepared and compounds were obtained that were four times as active *in vivo* and thirty times as active *in vitro* as the original thioamide (4, 12–14). As these compounds had no greater toxicity for laboratory animals than the original thioamide it was believed that they should be effective in the treatment of tuberculosis in humans at a dosage level that would be tolerated. Of these compounds the alpha-ethyl derivative, designated by the laboratory code number 1314 Th, appeared to be the most interesting and was chosen for the subsequent extensive studies (*see* table 1).

OBSERVATIONS

As a detailed presentation of certain of the observations has been made elsewhere (4, 15, 16), certain of the findings are mentioned only in brief in association with the most recent observations on this thioamide.

Activity in vitro: It was found that the thioamide was inhibitory for *M. tuberculosis* in Youmans medium with bovine serum in concentrations ranging between 0.6 and 1.2 γ per ml. This represents approximately one-tenth the activity of isoniazid in these circumstances and a degree of activity approximately the same as that of streptomycin. Moreover, as mentioned above, the thioamide, like isoniazid, caused the bacilli to lose their acid-fastness and was highly bactericidal under the conditions of the test.

Tubercle bacilli of bovine origin, including BCG, were found to be susceptible to 5 γ of the thioamide per ml. *M. balnei* was susceptible to 20 to 40 γ per ml., and *M. avium* to 40 γ per ml. In general, the scotochromogenic Mycobacteria were found to be susceptible to 20 to 40 γ per ml. and the photochromogenic Mycobacteria were found to be approximately as susceptible to the thioamide as were tubercle bacilli of human origin.

When tested in Tween®-albumin medium and on a solid medium of Löwenstein-Jensen, the inhibitory concentrations of the thioamide were two to four times greater than were those

observed in the Youmans medium with the added serum.

Activity in mice: Mice infected with tubercle bacilli of human origin were fully protected by the thioamide in a daily dosage of 12 mg. per kg. This dosage level is approximately ten times higher than the protective dose of isoniazid and eight times lower than the protective dose of streptomycin in this experimental model. When treatment with the thioamide was postponed until the infection had progressed for fourteen days, the bactericidal effect of the thioamide as demonstrated by the cultivation of the bacillary population in the mouse lungs was just as rapid as that observed with isoniazid and was considerably more rapid than that observed with streptomycin in the same experimental model (16).

Activity in the guinea pig: The present writers believe that the tuberculous infection in the mouse is relatively less susceptible to streptomycin than is the case with tuberculosis in humans. For this reason it was believed that, in order to make a comparison of the thioamide with streptomycin, observations should be made on experimentally infected guinea pigs. It was found that, in guinea pigs infected with tubercle bacilli of human origin, a daily dosage of 10 mg. per kg. of the thioamide was effective both when started simultaneously with infection and when chemotherapy was delayed for thirty-five days. On the basis of comparing the various dosage levels employed, it is inferred that the thioamide is twice as active as streptomycin and one-tenth as active as isoniazid in the guinea pig experimental system employed.

Toxicity of the thioamide in mice: When studies of the toxicity of the thioamide in mice were conducted, it was found that the LD_{50} was 1 gm. per kg. when the compound was administered by mouth. A daily dosage of 250 mg. per kg. by the oral route was well tolerated.

Absorption and distribution in man: After ingestion in human subjects, the thioamide was readily absorbed. Three, six, and nine hours after ingestion of 0.5 gm. of the compound, antimicrobially active serum concentrations of 12, 6, and 3 γ per ml. were observed. In the urine, only 1 per cent of the ingested drug was generally found in a state in which it was active *in vitro* against tubercle bacilli.

Tubercle bacilli resistant to the thioamide: Strains of tubercle bacilli resistant *in vitro* to thioamide concentrations of 10 to 20 γ per ml. can easily be obtained either by successive transfers *in vitro* or by experiments in mice. In the mouse, the procedure used was to start the thioamide treatment twelve to fourteen days after infection with tubercle bacilli and to isolate the bacilli from the lung after sixty to one hundred days of treatment. Previous experience with this technique had shown that it is possible in this manner to isolate tubercle bacilli resistant to isoniazid or to streptomycin (18). In multiple drug regimens consisting of the thioamide and isoniazid and the thioamide together with streptomycin or isoniazid and streptomycin, the appearance of the drug-resistant tubercle bacilli is avoided and the numbers of tubercle bacilli present in the lungs after four months of treatment are very few (16).

Strains of tubercle bacilli resistant *in vitro* to the thioamide have a normal catalase activity and a normal virulence for the guinea pig.

Cross-resistance with other antituberculous compounds: Strains of tubercle bacilli resistant *in vitro* to the thioamide are wholly susceptible to isoniazid *in vitro*. Strains resistant to PAS, streptomycin, viomycin, or cycloserine are likewise fully susceptible to the thioamide when tested *in vitro*.

The question of the effectiveness of the thioamide on tubercle bacilli resistant to isoniazid has been studied in detail and the results may be seen in table 2. In order to bring out slight differences in the drug susceptibilities, the minimal inhibitory concentrations of the thioamide in Youmans medium with serum was determined not at twelve days, but eighteen days after inoculation with 0.01 mg. of bacilli (moist weight). As may be seen in table 2, the proportion of strains of tubercle bacilli highly susceptible to the thioamide, i.e., susceptible to thioamide concentrations of 0.6 to 1.2 γ per ml., appeared to be greater among the strains that were highly resistant to isoniazid than among the strains that were wholly susceptible or only slightly resistant to isoniazid.

Four strains of tubercle bacilli have been isolated which, even before exposure to the thioamide, were found to be susceptible only to thioamide concentrations of 10 to 20 γ per ml. This primary resistance did not seem to be associated with resistance to isoniazid. The one strain that was resistant to thioamide and likewise resistant to an isoniazid concentration of

TABLE 2

SUSCEPTIBILITY TO α-ETHYL-THIOISONICOTINAMIDE OF 172 STRAINS SUSCEPTIBLE OR RESISTANT TO ISONIAZID

(Tested in Youmans-serum medium. Readings on the eighteenth day)

Minimal Inhibitory Concentration of α-Ethyl Thioamide (γ per ml.)	0.6	1.2	2.5	5	10	20	40
	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent
Isoniazid-susceptible, 87 strains....	0	30	65	4	0	1	0
Resistant to 0.15–5 γ of isoniazid, 53 strains.....	0	36	49	11	2	2	0
Resistant to ⩾10 γ of isoniazid, 32 strains.....	12.5	44	37	3	3	0	0

10 γ per ml. was isolated from a patient who had received large doses of thiosemicarbazone. In the other 3 instances it has not yet been possible either to confirm or to exclude the possibility that the patient had received previous treatment with thiosemicarbazone.

This question of the relation between resistance to the thioamide and to amithiozone has not yet been clearly defined. Strains of tubercle bacilli resistant to the thioamide after successive transfers *in vitro* have usually been found to be resistant to amithiozone and to the thiocarbanilides. In contrast, strains of tubercle bacilli resistant to amithiozone after *in vitro* successive transfers have been found to retain almost always the full susceptibility to the thioamide. It is of interest that α-ethyl-thioisonicotinamide, the thiosemicarbazones, and the thiocarbanilides all have the common grouping $CSNH_2$.

SUMMARY

Observations have been presented on the activity *in vitro* and in experimental animals of alpha-ethyl-thioisonicotinamide. Although this compound is a derivative of isonicotinic acid, it is not a derivative of isoniazid. Moreover, it is highly active both *in vitro* and *in vivo* on strains of tubercle bacilli that are resistant to isoniazid. The α-ethyl-thioisonicotinamide is four times more active than thioisonicotinamide, but has no greater toxicity than this compound. The type of action of α-ethyl-thioisonicotinamide is qualitatively similar to that of isoniazid. In quantitative terms, the thioamide is approximately twice as active as streptomycin and one-tenth as active as isoniazid in experimentally infected guinea pigs. The acute toxicity of thioamide for mice is only one-fifth of that of isoniazid. When administered by the oral route to man the thioamide has been found in the serum in a biologically active form. It was concluded, therefore, on the basis of these studies that the compound should receive clinical trials.

SUMARIO

Actividad Antituberculosa Experimental de la Alfa-etil-tioisonicotinamida

Estas observaciones versan sobre la actividad *in vitro* y en los animales de experimentación mostrada por la alfa-etil-tioisonicotinamida. Aunque este compuesto es un derivado del ácido isonicotínico, no es un derivado de la isoniacida. Sin embargo, es sumamente activo tanto *in vitro* como *in vivo* en las cepas de bacilos tuberculosos que son resistentes a la isoniacida. La α-etil-tioisonicotinamida es cuatro veces más activa que la tioisonicotinamida, pero no posee mayor toxicidad que este compuesto. La forma de la acción de la α-etil-tioisonicotinamida es cualitativamente semejante a la de la isoniacida. En términos cuantitativos, la tioamida es unas dos veces más activa que la estreptomicina y diez veces menos activa que la isoniacida en los cobayos infectados experimentalmente. La toxicidad aguda de la tioamida para los ratones es solamente la quinta parte de la isoniacida. Al administrarla por vía oral al hombre se descubrió en el suero en forma biológicamente activa. Se dedujo, por lo tanto, a base de estos estudios que el compuesto debe ser objeto de pruebas clínicas.

RESUME

Activite antituberculeuse experimentale de alpha-éthyl-thioisonicotinamide

Les propriétés antituberculeuses expérimentales de l'α-éthyl-thioisonicotinamide, *in vitro* et chez l'animal, ont été résumées. Bien que ce composé soit un dérivé de l'acide isonicotinique, il n'est pas un dérivé de l'isoniazide, et il est très actif sur les souches isoniazido-résistantes de bacilles tuberculeux, aussi bien *in vitro* que *in vivo*. L'α-éthyl-thioisonicotinamide est quatre fois plus actif *in vivo* que le thioisonicotinamide, mais il n'est pas plus toxique que ce dernier. Qualitativement, son action se rapproche de celle de l'isoniazide. Quantitativement, l'α-éthyl-thioisonicotinamide est, chez le cobaye tuberculisé, deux fois plus actif que la streptomycine et dix fois moins

actif que l'isoniazide. Sa toxicité aiguë chez la souris est cinq fois moindre que celle de l'isoniazide. Chez l'homme, administré par voie buccale, l'α-éthyl-thioisonicotinamide est retrouvé dans le sérum sous une forme biologiquement active. Cet ensemble de faits justifie son essai clinique.

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OBSERVATIONS ON THE ANTITUBERCULOUS EFFECTIVENESS OF ALPHA-ETHYL-THIOISONICOTINAMIDE IN TUBERCULOSIS IN HUMANS[1, 2]

G. BROUET, J. MARCHE, N. RIST, J. CHEVALLIER, AND G. LEMEUR

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INTRODUCTION

For the past year the writers have been carrying on clinical trials of the antituberculous effectiveness of the new compound alpha-ethyl-thioisonicotinamide. The synthesis of this compound was made by Liberman (1) and the biologic studies were carried on by Rist, Grumbach, Moyeux, Cals, Rouaix, and Clavel (2–5). The results of these studies are summarized in a previous report (6).

On the basis of the experimental studies by Rist and his associates (6), it was concluded that α-ethyl-thioisonicotinamide should be studied in man, particularly in patients with pulmonary tuberculosis that has not yielded satisfactorily to treatment with the conventional antimicrobial drugs. Accordingly, clinical trials with the α-ethyl-thioisonicotinamide were initiated in order to study its activity in tuberculosis in humans and to attempt to define whether this compound would play a role in tuberculosis chemotherapy.

GENERAL PLAN OF INVESTIGATION

The initial trials with the α-ethyl-thioisonicotinamide were started in the spring of 1957, but during the first several months only a very small number of patients were treated.

The α-ethyl-thioisonicotinamide was administered orally in the form of tablets, each containing 0.25 gm. of the drug. The usual regimen was 1 gm. per day or four tablets given either as two tablets twice daily or in the individual dosage of 0.25 gm. at six-hour intervals throughout the day. In some patients the appearance of digestive intolerance made it impossible to administer more than three tablets (0.75 gm.) of drug per day. All of the observations were made in the hospital under strict medical supervision.

Before the start of treatment each patient was given a complete examination consisting of a complete physical examination and careful studies of the sputum and gastric washings for tubercle bacilli. The various secretions were studied by both microscopy and culture if no acid-fast bacilli were seen on microscopy. All strains of tubercle bacilli isolated were tested *in vitro* for susceptibility to the thioamide, to isoniazid, and to other common antimicrobial drugs. Chest roentgenograms, including tomograms, were likewise obtained.

All of these observations were repeated regularly at four-week intervals on all patients throughout the entire period of treatment.

In addition, the patients were also examined once a week, and supplementary tests were carried out at fortnightly intervals to be sure that there were no toxic effects evident in the kidneys, liver, or the hemopoietic system.

The α-ethyl-thioisonicotinamide was administered to 102 patients, 79 of whom received the treatment for three months or more. Three patients received treatment for an entire year. The treatment was stopped prematurely (before the expiration of three months) in 23 cases either for reasons beyond the control of the writers (patient left the hospital, 12 cases; inadequate supply of drug, 3 cases) or by medical decision (drug intolerance, 5 cases; apparent ineffectiveness after two months' treatment, 3 cases).

Among the 102 patients treated were 73 men and 29 women. Fifty-seven of the patients had recently developed pulmonary tuberculosis, and in 45 cases this disease appeared to be old. All of the pulmonary lesions were classified in three categories according to extent (table 1).

Among the 79 patients who have received a minimum of three months' treatment with α-ethyl-thioisonicotinamide, one case has been excluded from analysis, as subsequent studies appeared to indicate that the patient had nontuberculous healed pulmonary disease.

There remains, therefore, for therapeutic evaluation a total of 78 patients, 43 with apparently recent disease and 35 with relatively chronic pulmonary tuberculosis (table 2). As in previous studies from this clinic, the cases have been classified according to age and severity of the lesions. It is believed essential to make the distinction between *recent lesions*, that is, lesions which apparently have evolved six months from the start of treatment, and *old lesions*, i.e., those that have been known to be present for more than six months.

Roentgenographic Classification

For purposes of analysis the lesions were classified into the following three groups: limited, when

[1] From L'Hôpital Cochin, Paris XIV^e^, France.

[2] Presented in part at the Scientific Sessions of the International Union Against Tuberculosis, Paris, July, 1958.

TABLE 1

CLASSIFICATION OF THE PATIENTS ACCORDING TO THEIR SEX AND THE AGE AND SEVERITY OF THE PULMONARY TUBERCULOUS LESIONS

Sex and Number of Cases	Recent Tuberculosis			Chronic Tuberculosis		
	Limited	Moderately Extensive	Extensive	Limited	Moderately Extensive	Extensive
Male, 73 cases	14	22	11	6	6	14
Female, 29 cases	1	7	2	—	7	12
Total, 102 cases	15	29	13	6	13	26
	57 cases			45 cases		

TABLE 2

DISTRIBUTION OF PATIENTS ACCORDING TO VARIOUS REGIMENS EMPLOYED

Recent Tuberculosis 43 Patients		Chronic Tuberculosis 35 Patients	
α-Ethyl-thioisonicotinamide Alone	α-Ethyl-thioisonicotinamide with Other Antimicrobials	α-Ethyl-thioisonicotinamide Alone	α-Ethyl-thioisonicotinamide with Other Antimicrobials
23 patients (*Cases 8–30*)	20 patients (*Cases 38–57*)	3 patients (*Cases 59–61*)	32 patients (*Cases 70–101*)

less than 20 per cent of the total pulmonary parenchyma was affected; moderately extensive, when the affected area corresponded to 20 to 40 per cent of the lungs; and extensive, when the tuberculosis affected more than 40 per cent of the parenchyma.

Although these designations are only approximate, an evaluation of this type appears valid when it is carried out by the same observers examining a large number of roentgenograms regularly. In the writers' experience this classification has made it possible to group the more common forms of pulmonary tuberculosis more easily according to their severity and development. The distinction between unilateral and bilateral lesions appeared unnecessary, as this factor does not come into play in the immediate prognosis of tuberculosis treated with antimicrobial drugs.

OBSERVATIONS

As the initial trials were pilot in nature, the choice of patients and the regimens employed had no preselected systematic character. It must not be forgotten that the antituberculous drugs in present use, particularly isoniazid, are highly effective in the majority of recent cases provided that the treatment is well directed and is maintained for a sufficient period. Moreover, because of the conviction that multiple-drug therapy is essential in the treatment of recent pulmonary tuberculosis in an attempt to avoid the emergence of drug-resistant strains of tubercle bacilli, trials of single-drug therapy can be conducted only with great care and to a limited extent in order not to endanger the patient's future.

For these reasons α-ethyl-thioisonicotinamide was administered *alone* to only 23 patients with recently diagnosed pulmonary tuberculosis. To 8 additional patients with recent disease which appeared more seriously progressive, the thioamide was administered along with isoniazid; and to 12 patients, streptomycin, isoniazid, and the thioamide were all given together. The isoniazid dosage employed was the one usually used on this service, namely 8 mg. per kg. per day, and the streptomycin, when given, was administered daily in a single dose of 1 gm.

Another group of patients consisted of those with pulmonary tuberculosis of long standing for which various antimicrobial drugs had been administered for long periods. In these cases the α-ethyl-thioisonicotinamide was given along with one or more other antituberculous drugs. The choice of the companion drug was made on the basis of drug susceptibility tests made *in vitro*.

A total of 32 patients received the thioamide as part of such multiple-drug regimens. Sixteen of these patients had extensive, persistently severe lesions which produced a steady emission of tubercle bacilli generally resistant to isoniazid or to streptomycin. In three highly specialized circumstances, patients with similarly chronic lesions were treated with the thioamide alone. The distribution of the patients among the various regimens may be seen in table 2.

Drug Toxicity

Gastric intolerance: It was found that the α-ethyl-thioisonicotinamide was frequently badly tolerated, but was not associated with severe toxicity even when it was administered for periods of three months or more. The toxicity studies in animals are presented in a previous paper (6).

In the present studies with 102 patients, it was found virtually impossible to administer more than 1 gm. of α-ethyl-thioisonicotinamide daily

to patients weighing 60 to 70 kg. On the average, the maximally tolerated daily dosage represented approximately 15 mg. per kg. Many attempts were made to increase this daily dosage, but all failed because of serious gastric intolerance, and only very few patients could comfortably receive six tablets a day for any continued period. Accordingly, it is believed that the daily dosage can be fixed at 15 mg. per kg., i.e., 4 of the 0.25-gm. tablets. Even at this dosage level, the gastric tolerance was often poor, and digestive disorders were frequent.

Anorexia was the most common symptom. At times it appeared only as a temporary disorder, manifested by a loss of appetite and a failure to gain weight. In many patients the anorexia was transient, and after two to four weeks of treatment the appetite returned and was accompanied by a slow gain in weight. Less frequently the anorexia remained persistent or even increased, and a real revulsion for food appeared. Substantial weight losses were then observed. In *Case 10*, the weight loss amounted to 5 kg.; and in *Case 24*, to 6.5 kg. In addition to the anorexia, the patients often complained of a "feeling ill" sensation which disappeared promptly when the treatment was discontinued. The anorexia was often associated with an intermittent nausea and gastric irritation. In some patients, however, the gastric irritation and nausea were persistent and became extremely distressing and were accompanied by vomiting. This occurred in the 5 cases of severe intolerance observed, which necessitated cessation of treatment. To some extent it was found that, when the α-ethyl-thioisonicotinamide was administered during meals and the tablet had a glutinized coating, the tolerance seemed to be a little better.

An attempt was made to define the incidence of gastric intolerance by separating the 102 patients into five groups under the headings of *good tolerance*, *moderate tolerance*, *severe intolerance* (a strong and lasting anorexia with a failure to gain weight), *very severe intolerance* (persistent anorexia with gastric irritation and nausea), and *complete intolerance* (symptoms similar to the others but of such severity that it was necessary to discontinue treatment).

As may be seen from table 3, 61 patients were able to receive the drug regularly with no major inconvenience. In 29 patients, however, the degree of gastric intolerance was classified as severe, and in 7 it was very severe. In 5 cases, intolerance was complete, leading to cessation of treatment.

TABLE 3
GASTRIC INTOLERANCE

Good Tolerance	Moderate Tolerance	Severe Intolerance	Very Severe Intolerance	Complete Intolerance
55 patients	6 patients	29 patients	7 patients	5 patients
61			12	

Consequently, gastric intolerance of a serious nature was found in 12 per cent of the cases. Except for the obvious distress occasioned to the patient and the necessity for discontinuing treatment in the more severe instances, these digestive phenomena were not associated with any evidences of potentially serious drug toxicity.

Hepatic function: None of the patients developed any clinical evidence of icterus. Moreover, studies of hepatic function conducted regularly revealed no abnormalities.

Renal function: In all treated patients, the test for albumin and the examination of urinary sediment were carried out regularly twice a month. The results were always normal.

Hematotoxic effects: There was no bleeding in any of the patients. Blood counts performed every fortnight showed no anemia or leukopenia; in particular, a decrease in the number of granulocytes was never observed.

Neurotoxic effects: In 2 patients treated with α-ethyl-thioisonicotinamide in association with cycloserine, convulsions were observed. In a patient who had already received isoniazid in high dosage for a great length of time, symptoms of polyneuritis appeared during combined treatment with isoniazid and α-ethyl-thioisonicotinamide. In these 3 cases, the toxic role of α-ethyl-thioisonicotinamide cannot be denied. In a patient who suffered from neuritis symptoms during treatment with isoniazid, interruption of the treatment was followed by great improvement, while re-treatment with α-ethyl-thioisonicotinamide resulted, within three days, in the reappearance of neuritic disorders which likewise disappeared on stopping the new drug.

The tests carried out on 102 patients, 79 of whom were treated for more than three months, make it possible to conclude that the drug has

no major toxic effects in man in the dosage used; the main problem remains that of digestive tolerance.

Therapeutic Effectiveness

The influence of the α-ethyl-thioisonicotinamide on the tuberculous process, as judged by the clinical observations, appeared to be generally comparable with the results seen from the use of streptomycin or isoniazid. The reduction in expectoration, however, occurred less rapidly than is usually seen with isoniazid. Nevertheless, the influence of the thioamide was frequently clearly visible after administration for a two- to four-week period. In several cases defervescence occurred rapidly after the start of the thioamide treatment and was associated with a return to normal of the values for the erythrocyte sedimentation.

The α-ethyl-thioisonicotinamide did not appear comparable with isoniazid in terms of its action on the general status of the patients. A rapid change in appetite and important weight increases occurred only rarely in contrast to the situation usually found from the use of isoniazid-streptomycin therapy. The poor or even bad effects of the drug on the general status of the patient could be seen most clearly from the weight charts, because all the patients were maintained on bed rest in the hospital throughout the period of observation.

Of the 23 patients with recent pulmonary tuberculosis who were treated with the thioamide alone for a three-month period, 6 lost weight, one failed to increase in weight, and 16 showed weight gains. These data are presented in table 4. Thirteen of the patients gained weight only during the first month of treatment; and for the 16 patients whose weight did increase, the average increase after three months of therapy was 2.7 kg. Moreover, when the thioamide was given in association with isoniazid, the beneficial effect of the latter drug on general well-being usually observed was not perceptible. In these cases, however, if the thioamide was discontinued, a rapid and favorable change in well-being occurred followed by a rapid weight increase. For example, in one patient whose weight had remained stable at 38 kg. during treatment with both drugs, a gain of 1.5 kg. in the first month and 2.5 kg. in the second month occurred after cessation of the thioamide therapy.

TABLE 4

MONTHLY AND END-OF-TREATMENT WEIGHT CHANGES IN 23 PATIENTS WITH RECENT TUBERCULOSIS TREATED WITH α-ETHYL-THIOISONICOTINAMIDE ALONE

Periods	Loss of Weight	No Change in Weight	Increase in Weight
First month	5	5	13
Second month	6	3	14
Third month	7	5	11
At end of 3 months	6	1	16

Comment: It is important that the question of gastric intolerance and failure to improve in well-being be neither overstressed nor underemphasized. The writers believe that a general improvement and progressive weight increase remain favorable indices in phthisiotherapy. It must also be remembered that an improvement in general feeling, especially at the start of treatment, has usually quite a beneficial psychologic effect on patients and leads to their having a very real confidence in the effectiveness of the drugs that they are receiving. It is believed, therefore, that these factors must be considered with respect to the α-ethyl-thioisonicotinamide and, except in cases of absolute necessity, it is believed that the drug should not be given for longer than four to six weeks to patients who are definitely having symptoms of intolerance.

Roentgenographic Studies

Because of the limited nature of the trial of the α-ethyl-thioisonicotinamide, the lesions have not been evaluated quantitatively, as has been done in the previous prolonged studies from this clinic. The principal reason for this is that the quantitative method is only of value with data obtained over a six-month period. Consequently, the evaluations are essentially subjective, but they are the result of a collective examination of the roentgenograms by a team accustomed to working together and whose individual evaluations are usually in accord.

The roentgenographic results have been classified as follows:

No improvement: when the lesions remain unchanged.
Moderate improvement: when the improvement is definite but the final outcome appears unpredictable.
Substantial improvement: when the improve-

ment is not only substantial, but the final outcome appears almost certainly favorable.
Marked improvement: when the action on nodules and cavities suggests an early cure.

The roentgenographic observations presented for all of the patients are those noted after a three-month period of treatment with the thioamide.

The results have been presented for three separate groups in order to distinguish between the limited, moderately extensive, and extensive lesions.

Recent tuberculosis treated by α-ethyl-thioisonicotinamide alone: The results observed in the 23 patients who received only the thioamide are presented in table 5. Substantial and marked improvement was noted in 4 of the 9 cases of limited disease and in 4 of the 12 of moderately extensive disease, or in a total of 8 of the 23 patients. These observations are concerned with only a small number of patients, but, in agreement with the bacteriologic data, show an unquestioned activity of the compound in tuberculosis in man.

Recent tuberculosis treated by α-ethyl-thioisonicotinamide in association with isoniazid or with isoniazid and streptomycin: In 20 patients, most of whom had more extensive disease than the previous group, isoniazid (8 patients) or isoniazid and streptomycin (12 patients) were administered along with the thioamide (table 6). In this group, substantial or marked improvement was observed in 9 patients. This result is not very different from that observed after three months in patients with a similar type of disease treated by isoniazid alone, or in multiple-drug treatment with streptomycin and PAS. The substantial or marked improvement in 9 of 20 patients is little higher than was observed in the patients in the present study who received the thioamide alone. It was not possible, however, to attach significance to this numerical difference since each group represents such a small number of patients.

TABLE 5

ROENTGENOGRAPHIC RESULTS AFTER 3 MONTHS IN THE 23 CASES OF RECENT PULMONARY TUBERCULOSIS TREATED WITH α-ETHYL-THIOISONICOTINAMIDE ALONE

Categories	Number of Cases	Roentgenographic Results				
		No improvement	Slight Improvement	Moderate Improvement	Substantial Improvement	Marked Improvement
Limited tuberculosis	9	2	1	2	3	1
Moderately extensive tuberculosis	12	1	2	5	3	1
Extensive tuberculosis	2		1	1		
Total	23	3	4	8	6	2

TABLE 6

ROENTGENOGRAPHIC RESULTS AFTER 3 MONTHS IN THE 20 CASES OF RECENT PULMONARY TUBERCULOSIS TREATED WITH α-ETHYL-THIOISONICOTINAMIDE AND ISONIAZID (OR ISONIAZID AND STREPTOMYCIN)

Categories	Number of Cases	Roentgenographic Results				
		No improvement	Slight Improvement	Moderate Improvement	Substantial Improvement	Marked Improvement
Limited tuberculosis	1	1*				
Moderately extensive tuberculosis	10			4	5	1
Extensive tuberculosis	9	1	1	4	3	
Total	20	1*	1	8	8	1

* No interpretation possible.

TABLE 7

ROENTGENOGRAPHIC RESULTS AFTER 3 MONTHS IN THE 32 CASES OF CHRONIC PULMONARY TUBERCULOSIS TREATED WITH α-ETHYL-THIOISONICOTINAMIDE IN ASSOCIATION WITH OTHER ANTITUBERCULOUS DRUGS

Categories	Number of Cases	Roentgenographic Results				
		No improvement	Slight Improvement	Moderate Improvement	Substantial Improvement	Marked Improvement
Limited tuberculosis	6	2	2	1		
Moderately extensive tuberculosis	10		6	3	1	
Extensive tuberculosis	16	8	2	3	2	1
Total	32	10	10	7	3	1

Chronic tuberculosis treated with α-ethyl-thioisonicotinamide in association with other antituberculous drugs: In the group of patients with chronic lesions treated extensively with the customary antituberculous drugs, it was not anticipated that remarkable roentgenographic improvement would be observed during thioamide therapy (table 7). Nevertheless, it is of interest that among 26 patients with moderately extensive or extensive chronic disease, substantial roentgenographic improvement occurred in 3 patients and marked improvement occurred in one patient. As will be shown later, bacteriologic results in certain of these patients were likewise most satisfactory and presumably represented the influence of the thioamide.

Bacteriologic Studies

Tubercle bacilli were demonstrated in the discharges of 86 of the 101 patients at the time treatment with the α-ethyl-thioisonicotinamide was started.

The drug-susceptibility tests were carried out in Youmans medium. Two types of inocula were used. One consisted of 0.05 ml. of bacilli (wet weight) taken from Jensen medium, suspended in Tween®-albumin medium, and incubated for forty-eight hours in the oven. This is the technique employed in the laboratory at l'Hôpital Cochin. The other inoculum consisted of 0.01 ml. bacilli (wet weight) obtained from a culture growing in Tween-albumin medium that was well homogenized and had attained full growth. This is the technique employed at the Institut Pasteur. The weights were measured by optic density and the inocula represented on the average 100,000 to 500,000 viable units of tubercle bacilli. The tests were interpreted on the sixth, twelfth, and eighteenth days and the results from both techniques showed good agreement.

The total resistance of a strain is given in terms of the tube in which, after the sixth day, the growth equals that observed in the control tube. The total susceptibility is recorded as the tube in which there was either no growth or only a very sparse growth, approximately 1 to 200 colonies, after eighteen days. If it be assumed that each colony develops from a single bacillus, the presence of only a few colonies shows that, of the 100,000 inoculated, only one to 200 bacilli were capable of reproducing in the tube. In other words, almost the total number of bacilli in the population tested was inhibited by the corresponding concentration of the drug. A strain is best characterized by presenting both its total resistance and total susceptibility, but in most cases only the total susceptibility is presented.

According to these definitions, the strains of the untreated patients fell in the following limits:

	Total Susceptibility
Isoniazid	0.04 to 0.15 γ
Streptomycin	0.6 to 1.2 γ
PAS	0.6 to 1.2 γ
Viomycin	5 to 10 γ
Cycloserine	10 to 15 γ
α-Ethyl-thioisonicotinamide	1.2 to 2.5 γ

With 83 strains it was possible to test immediately and simultaneously the susceptibility to both α-ethyl-thioisonicotinamide and to isoniazid. The drug-susceptibility tests for streptomycin, PAS, cycloserine, and viomycin were studied on the strains from a number of patients, mainly those with the more chronic lesions (table 8). Consequently it was possible to note that without exception strains of *M. tuberculosis*, resistant to the other drugs, were normally susceptible to the thioamide. The strains of tubercle bacilli isolated from 80 of the 83 patients at the time when treatment was started showed susceptibility to the α-ethyl-thioisonicotinamide of 1.2 to 2.5 γ per ml. Only 3 strains, isolated from 2 patients with recent disease and one with chronic disease represent an exception in that the initial susceptibility was between 5 and 10 γ of the thioamide per ml. No obvious explanation has been found for this finding. The results of the thioamide-susceptibility test are presented diagrammatically in figure 1. The lack of any cross-resistance with respect to isoniazid and α-ethyl-thioisonicotinamide may be seen from the data presented in table 9.

Recent tuberculosis treated by α-ethyl-thioisonicotinamide alone: In the group of 23 patients who received the thioamide alone, 18 were discharging tubercle bacilli at the start of treat-

TABLE 8

NUMBER OF DRUG-SUSCEPTIBILITY TESTS PERFORMED BEFORE TREATMENT WITH α-ETHYL-THIOISONICOTINAMIDE

	Result		α-Ethyl-thioisonicotinamide	Isoniazid	Streptomycin	PAS	Cycloserine	Viomycin
	0	+						
Recent disease (57 cases)	9	48	47	47	24	12	1	1
Chronic disease (44 cases)	6	38	36	36	34	27	21	19
Total (101 cases)	15	86	83	83	58	39	22	20

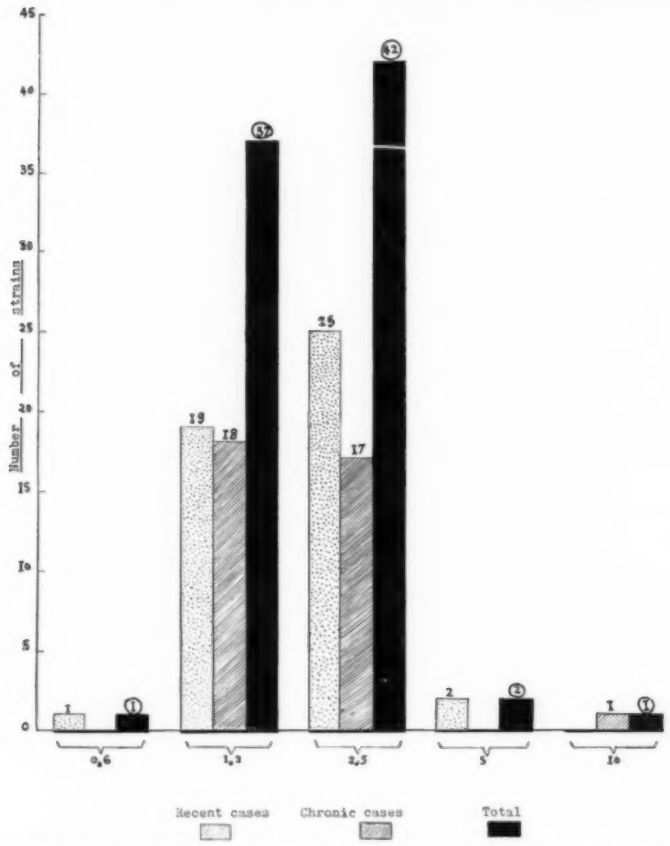

FIG. 1. Susceptibility to α-ethyl-thioisonicotinamide (expressed in γ per ml. in Youmans medium with beef serum added) of 83 strains of *M. tuberculosis* isolated before treatment.

ment (table 10). At the end of the treatment, the 5 patients whose sputum was not infectious at the beginning remained in the noninfectious state, although in 2 cases tubercle bacilli had appeared in the sputum at some time or other during the treatment. Of the 18 patients whose sputum was positive for tubercle bacilli at the beginning of the treatment, only 7 were still discharging bacilli after three months of treatment; and, in the other 11, tubercle bacilli could no longer be isolated from the sputum.

The group of 12 patients with moderately extensive lesions was of particular interest. Six of the 11 patients of this group with positive bacteriologic findings showed reversal of infectiousness by the end of treatment. This is interpreted as showing, unequivocally, the degree of effectiveness of the α-ethyl-thioisonicotinamide on pulmonary tuberculosis. It should also be noted that the bacilli in 3 of the 5 patients whose infectiousness persisted showed a loss of susceptibility to the α-ethyl-thioisonicotinamide.

This loss of drug susceptibility can appear early, and in one case appeared at two months and in 2 other cases appeared after only one month of therapy. The early appearance of drug resistance must be emphasized because it appeared to correspond to treatment failure. In 3 patients (*Cases 4, 6,* and *7*, which are not included in table 10) the treatment was discon-

TABLE 9

COMPARATIVE STUDY OF THE SUSCEPTIBILITY TO ISONIAZID AND α-ETHYL-THIOISONICOTINAMIDE OF 83 STRAINS ISOLATED BEFORE TREATMENT WITH α-ETHYL-THIOISONICOTINAMIDE (Given in γ per ml.) IN YOUMANS MEDIUM WITH BEEF SERUM ADDED

Categories	Susceptibility to α-Ethyl-thio-isonicotinamide	Susceptibility to Isoniazid														
		0.04	0.08	0.1	0.15	0.2	0.25	0.30	0.50	1	2	2.5	5	10	20	25	50
Recent cases (47)	0.6		1														
	1.2	1	13		4		1										
	2.5		2	3	15	2	1		1								1
	5.0			1				1									
	10.0																
Chronic cases (36)	0.6																
	1.2		2	1						1		1	2	3	2	2	4
	2.5			1	2				1		1		4	3	1	1	3
	5.0																
	10.0								1								
Total (83 cases)	0.6		1														
	1.2	1	15	1	4		1			1		1	2	3	2	2	4
	2.5		2	4	17	2	1		2		1		4	3	1	1	4
	5.0			1				1									
	10.0								1								

TABLE 10

BACTERIOLOGIC CHANGES IN 23 PATIENTS WITH RECENT PULMONARY TUBERCULOSIS TREATED WITH α-ETHYL-THIOISONICOTINAMIDE ALONE FOR THREE MONTHS

Categories	Limited Tuberculosis 9 Cases		Moderately Extensive Tuberculosis 12 Cases		Extensive Tuberculosis 2 Cases		Total 23 Cases	
Bacteriology	0	+	0	+	0	+	0	+
Initially	4	5	1	11	—	2	5	18
After 1 month	3(C+)*	3	1	6	—	2	4	11
After 2 months	4	2	0 (C+)	8	—	1	4	11
After 3 months	4	1	1	5	—	1	5	7

* C+ indicates that a patient with a culture initially negative for *M. tuberculosis* shows a positive culture.

tinued prematurely at the end of the second month because of complete lack of roentgenographic improvement and the persistence of large numbers of tubercle bacilli in the sputum. The drug-susceptibility tests obtained subsequently in these 3 cases revealed the fact that the strains had lost susceptibility to the thioamide without losing susceptibility to the isoniazid. Moreover, when the 3 patients were subsequently treated with orthodox antimicrobial therapy, a very rapid improvement was obtained.

On the basis of this experience, the writers have come to a practical conclusion that the thioamide should not be administered alone. The results presented below on the bacteriologic findings of the patients on multiple-drug therapy substantiate this conclusion.

Recent tuberculosis treated by α-ethyl-thioisonicotinamide in association with isoniazid or isoniazid and streptomycin: Only one of the group of 20 patients who received multiple-drug therapy, including the thioamide, was noninfectious at the beginning of treatment (table 11). In this case the noninfectious status was maintained throughout treatment. Only 2 of the 19 patients with tubercle bacilli in the sputum at the start of treatment were still discharging the bacilli at the end of treatment. This is considered

TABLE 11

BACTERIOLOGIC CHANGES IN 20 PATIENTS WITH RECENT PULMONARY TUBERCULOSIS TREATED WITH α-ETHYL-THIOISONICOTINAMIDE IN ASSOCIATION WITH ISONIAZID (OR ISONIAZID AND STREPTOMYCIN) FOR 3 MONTHS

Categories	Limited Tuberculosis 1 Case		Moderately Extensive Tuberculosis 10 Cases		Extensive Tuberculosis 9 Cases		Total 20 Cases	
Bacteriology	0	+	0	+	0	+	0	+
Initially	1	—	—	10	—	9	[1]	[19]
After 1 month	1	—	—	8	—	9	1	17
After 2 months	1	—	—	1	—	6	1	7
After 3 months	1	—	—	0	—	2	[1]	[2]

TABLE 12

BACTERIOLOGIC CHANGES IN 16 PATIENTS WITH CHRONIC, LIMITED, OR MODERATELY EXTENSIVE PULMONARY TUBERCULOSIS TREATED WITH α-ETHYL-THIOISONICOTINAMIDE IN ASSOCIATION WITH DIFFERENT ANTIMICROBIAL DRUGS FOR 3 MONTHS

Categories	Limited Tuberculosis 6 Cases		Moderately Extensive Tuberculosis 10 Cases		Total 16 Cases	
Bacteriology	0	+	0	+	0	+
Initially	4	2	—	10	[4]	[12]
After 1 month	4	0	—	6	4	6
After 2 months	4	0	—	3	4	3
After 3 months	4	0	—	1	[4]	[1]

to be a remarkable result in view of the fact that it was obtained in such a relatively short period of time. This reversal of infectiousness in 17 of the 19 patients on the multiple-drug therapy was observed in those with moderately extensive lesions. It should also be noted that the 2 patients who failed to attain noninfectious status at the end of treatment had extensive lesions, and their strains of bacilli were still susceptible to both the thioamide and isoniazid at the end of the three-month period of treatment.

Concerning the 11 patients treated with α-ethyl-thioisonicotinamide in association with both isoniazid and streptomycin, the role of α-ethyl-thioisonicotinamide is difficult to evaluate. The 8 cases treated with α-ethyl-thioisonicotinamide in association with isoniazid alone are more interesting: 7 showed reversal of infectiousness within three months; the only strain of tubercle bacilli cultivated after three months was susceptible to isoniazid and to α-ethyl-thioisonicotinamide.

In this small series, the isoniazid-thioamide combination proved as efficient as the classic isoniazid-streptomycin combination, and the α-ethyl-thioisonicotinamide seems to have prevented the appearance of the resistance to isoniazid.

Chronic tuberculosis treated by α-ethyl-thioisonicotinamide in association with different antimicrobial drugs: It is necessary to divide the group of 32 patients with chronic disease who received multiple-drug therapy, including the thioamide, into two separate subgroups. The first is comprised of 16 patients with chronic disease, but of a relatively limited extent (table 12). The second group consists of 16 patients with more extensive disease which was frequently bilateral. In the first subgroup, 6 of the 16 patients had limited and sluggish lesions and 4 of them were without tubercle bacilli in the sputum. Reversal of infectiousness was demonstrated in the other 2 patients during the period of treatment. The other 10 patients in the first subgroup had moderately extensive lesions and all were discharging tubercle bacilli at the start of treatment. In 9 of these 10 patients tubercle bacilli disappeared from the sputum during the three-month period of treatment. This was considered to be particularly remarkable in view of the fact that the *in vitro* tests of these patient-strains revealed a loss of susceptibility to isoniazid and to streptomycin in most instances.

The second subgroup of 16 patients consisted of those with extensive lesions, and the patients had all been discharging tubercle bacilli for many months. In virtually all cases, the strains isolated were highly resistant to isoniazid, but in all cases were susceptible to the thioamide. The α-ethyl-thioisonicotinamide was used most often in association with cycloserine and viomycin in this group, and in 10 of the 16 patients reversal of infectiousness occurred in three or four months.

DISCUSSION

The present clinical trial with α-ethyl-thioisonicotinamide has shown that this drug has certain

interesting properties and, therefore, should have a part in phthisiotherapy.

Bacteriologic studies *in vitro* of 83 strains derived from the patients show definitely the activity of the thioamide. This activity was seen both on strains from previously untreated patients and on strains resistant *in vitro* to various other antimicrobial drugs, including isoniazid.

In the patients the antituberculous activity of the drug was likewise evident not only in the frequent reversal of infectiousness of the bronchopulmonary secretions during treatment, but also by the appearance in a certain number of patients of strains of tubercle bacilli resistant to the thioamide. In all cases encountered, the emergence of resistance to thioamide was not associated with the changes in susceptibility to isoniazid.

The roentgenographic observations during the three-month period of therapy are likewise evidence of definite activity of the thioamide on tuberculosis in humans. The over-all results at the end of treatment in 23 patients with recently diagnosed disease treated by the thioamide alone were satisfactory in 10 instances.

However, two points should be noticed:

(*1*) The drug is often badly tolerated, giving very troublesome digestive disorders and especially an intense anorexia which can lead to an important weight loss. If the minor intolerance symptoms often observed at the start of treatment give way rather rapidly, continuation of the drug is possible. If more serious disorders occur, however, bringing about a stabilization of weight or even a loss of weight, the treatment should be stopped unless the indications for continuance are extremely strong.

(*2*) The use of α-ethyl-thioisonicotinamide in association with isoniazid or cycloserine necessitates careful clinical supervision as a few neurotoxic symptoms have been observed during treatment with this two-drug regimen. It should be noted, however, that the role of α-ethyl-thioisonicotinamide was not clearly demonstrated in 3 of these 4 cases. As usual, the possibility of neurotoxicity should be especially feared among epileptics and alcoholics.

What may be said about the position of the thioamide in the treatment of recently diagnosed pulmonary tuberculosis? The experience of the writers since 1952 with prolonged antimicrobial therapies has shown that, in cases of recent tuberculosis, isoniazid must be considered to be the major drug. But isoniazid must be given immediately without interruption for periods of eighteen to twenty-four months. Moreover, it should be given in sufficient doses, 8 to 10 mg. per kg. daily. It also seems that these relatively large doses delay the appearance of resistant strains in serious cases in which reversal of infectiousness is not obtained in the first few months of treatment. The writers have, likewise, been convinced that multiple-drug therapies have a more rapid action, especially in serious or important cavitary tuberculosis. Consequently, it has been the practice, especially during the first few months of therapy, to use a triple-drug regimen of isoniazid, streptomycin, and PAS, the PAS being given intravenously. It is believed that this triple-drug regimen is more active than the isoniazid-PAS regimen.

On the basis of the present experience with the α-ethyl-thioisonicotinamide, it appears that it too should be administered as part of a multiple-drug regimen. In several patients who received the thioamide alone and showed no improvement, the addition of isoniazid alone, or together with streptomycin, brought about rapid roentgenographic improvement and disappearance of tubercle bacilli from the sputum. Moreover, in these patients the bacilli had usually shown the appearance of thioamide resistance. In the cases in which relatively rapid bacterial resistance to the thioamide appeared, it is of considerable interest that the strains continued to be susceptible to isoniazid.

It is evident, therefore, that the α-ethyl-thioisonicotinamide must never be administered alone as a major treatment for pulmonary tuberculosis, and that it should be used along with a sufficient dose of isoniazid as part of multiple-drug therapy. It is not yet possible to state whether the α-ethyl-thioisonicotinamide could be advantageously substituted for streptomycin or PAS at some stage of the multiple-drug treatment.

At the moment it is believed, on the basis of the present experimental clinical investigations, that a study of the triple-drug regimen of isoniazid, streptomycin, and α-ethyl-thioisonicotinamide is permissible in order to determine more precisely its effectiveness in progressive pulmonary tuberculous lesions.

What may be said about the position of α-ethyl-thioisonicotinamide in the treatment of chronic pulmonary tuberculosis, already treated with classic drugs?

One of the most difficult therapeutic problems

today is in certain cases of chronic pulmonary tuberculosis that have not responded satisfactorily to treatment. In most cases, the lesions in these patients either existed before the days of antimicrobial therapy or are present in patients who interrupt their treatment as soon as they feel better and then suffer relapse of their disease. All of these chronically ill patients have extensive lesions, generally bilateral and with large cavities, and usually the patients consistently discharge tubercle bacilli resistant to both streptomycin and isoniazid. A certain number of these patients may be satisfactorily managed by a combination of medical re-treatment along with judiciously timed surgery. Cycloserine, viomycin, and α-ethyl-thioisonicotinamide in different regimens, according to the previous treatments received and to the bacteriologic data, are capable of producing reversal of infectiousness in a number of these patients. Thus, in certain forms of chronic tuberculosis that have not responded satisfactorily to classic treatments, there are certain drugs that are capable of exerting a considerable degree of effectiveness and α-ethyl-thioisonicotinamide is certainly among these compounds.

Summary

A clinical investigation has been made of the use of α-ethyl-thioisonicotinamide in 102 patients with pulmonary tuberculosis. In 78 of the patients the drug was administered either alone or with another antimicrobial drug for a period of three months or more. Forty-three of the 78 patients had relatively recent disease, and in the remaining 35 patients the tuberculosis was chronic.

The α-ethyl-thioisonicotinamide was often badly tolerated and gave rise to digestive disorders such as anorexia, nausea, or gastric irritation. These entailed either a cessation of weight gain or an actual loss of weight. There was no evidence that the drug was toxic for the liver, the kidneys, or the hematopoietic system. The neurotoxicity of α-ethyl-thioisonicotinamide appears to be minimal and requires special attention only in patients treated simultaneously with isoniazid or cycloserine.

The α-ethyl-thioisonicotinamide had an undeniable influence on the clinical, roentgenographic, and bacteriologic evidences of the pulmonary tuberculosis in the patients treated. The bacteriologic effects were particularly evident and, in patients with recent disease, reversal of infectiousness was obtained within a two- to three-month period in a high number of instances.

Within the period of observation the drug's action was not constant. A lack of improvement and the appearance of strains resistant to the α-ethyl-thioisonicotinamide were observed in several patients. In these cases, however, the tubercle bacilli had maintained susceptibility to other antimicrobials, and a change of treatment brought about favorable therapeutic results. It is believed, however, that the thioamide should not be used alone in the initial treatment of recently developed pulmonary tuberculosis, but should be administered in association with isoniazid. The results obtained when the thioamide was used in multiple-drug regimens, including isoniazid or isonazid and streptomycin, were considered to be most satisfactory. It is believed, therefore, that α-ethyl-thioisonicotinamide, as is the case with cycloserine and with viomycin, is a drug of great interest for the treatment of chronic, often serious, cases of pulmonary tuberculosis. When used in association with other drugs, the thioamide has brought about considerable improvement of the lesions and reversal of infectiousness in patients who had received much previous therapy but whose strains of tubercle bacilli were completely resistant *in vitro* to isoniazid and to streptomycin.

Sumario

Observaciones sobre la Eficacia Antituberculosa de la Alfa-etil-tioisonicotinamida en la Tuberculosis en los Seres Humanos

Esta investigación clínica versa sobre el empleo de la α-etil-tioisonicotinamida en 102 enfermos de tuberculosis pulmonar. En 78 de los sujetos se administró la droga ya sola o combinada con otra droga antimicrobiana durante un período de tres meses o más. Cuarenta y tres de los 78 tenían enfermedad relativamente reciente y en los otros 35 la dolencia era crónica.

La α-etil-tioisonicotinamida fué a menudo mal tolerada, produciendo trastornos digestivos, tales como anorexia, náuseas o irritación gástrica. Estos ocasionaron ya cese del aumento de peso o pérdida efectiva de peso. No hubo signos de que la droga fuera tóxica para el hígado, los riñones o el aparato hematopoyético. Sin embargo, al asociarse con la isoniacida o la cicloserina, pareció ser capaz de acrecentar la neurotoxicidad de las últimas drogas.

La α-etil-tioisonicotinamida ejerció influjo innegable sobre los signos clínicos, roentgeno-

gráficos y bacteriológicos de la tuberculosis pulmonar en los enfermos tratados. Los efectos bacteriológicos fueron en particular manifiestos y, en los sujetos con enfermedad reciente, se obtuvo viraje de la infecciosidad en un período de dos a tres meses en un número subido de casos.

En el período de observación, la acción de la droga no fué constante, notándose en varios enfermos falta de mejoría y aparición de cepas resistentes a la α-etil-tioisonicotinamida. No obstante, en estos casos los bacilos tuberculosos habían retenido su susceptibilidad a otros antimicrobianos y un cambio de tratamiento logró favorables resultados terapéuticos. Sin embargo, opínase que no debe usarse la tioamida por sí sola en el tratamiento inicial de la tuberculosis pulmonar reciente, sino que debe administrarse junto con la isoniacida. Los resultados obtenidos cuando se usó la tioamida en regímenes compuestos de varias drogas, incluso isoniacida o isoniacida y estreptomicina, se consideran de lo más satisfactorios. Parece, pues, que la α-etil-tioisonicotinamida, lo mismo que sucede con la cicloserina y la viomicina, es un medicamento de mucho interés para el tratamiento de casos crónicos, y a menudo graves, de tuberculosis pulmonar. Cuando se usa junto con otras drogas, la tioamida ha logrado considerable mejoría de las lesiones y viraje de la infecciosidad en enfermos que habían recibido mucha terapéutica anteriormente, pero cuyas cepas de bacilos tuberculosos eran totalmente resistentes *in vitro* a la isoniacida y la estreptomicina.

Resume

Observations sur l'efficacité anti-tuberculeuse de alpha-éthyl-thioisonicotinamide dans la tuberculose chez l'homme

Une investigation clinique sur l'emploi de α-éthyl-thioisonicotinamide a été entreprise chez 102 patients atteints de tuberculose pulmonaire. Chez 78 d'entre eux, la drogue a été administrée seule ou associée à un autre agent antibactérien pendant une période de trois mois ou plus. Parmi ces 78 malades, l'infection tuberculeuse était relativement récente chez 43 et chronique chez les 35 autres.

La α-éthyl-thioisonicotinamide était souvent mal tolérée et provoquait certains troubles digestifs, tels que l'anorexie, les nausées, ou l'irritation gastrique, ce qui entraînait un arrêt de l'augmentation pondérale ou même une perte de poids. Aucun signe de toxicité du médicament pour le foie, le rein ou le système hématopoïétique n'a été constaté. Toutefois, associée à l'isoniazide ou à la cyclosérine elle semble capable d'accroître la neurotoxicité de ces médicaments.

La α-éthyl-thioisonicotinamide exerce une influence indéniable sur les manifestations cliniques, radiologiques et bactériologiques de la tuberculose pulmonaire des malades traités. Les effets bactériologiques étant particulièrement évidents et, dans un grand nombre de cas, la négativation de la bacilloscopie a été obtenue dans les deux à trois mois.

Durant la période d'observation, l'action de la drogue n'était pas constante. Chez plusieurs malades l'amélioration manquait et on a observé l'apparition de souches résistantes à la α-éthyl-thioisonicotinamide. Dans ces cas, les bacilles tuberculeux conservaient néanmoins leur sensibilité à d'autres antibactériens et un changement de traitement a amené de bons résultats thérapeutiques. Toutefois, on estime que dans le traitement initial de la tuberculose pulmonaire d'apparition récente la thioamide ne doit pas être administrée seule, mais associée à l'isoniazide. Les résultats obtenus lorsque la thioamide était utilisée dans le traitement combiné comportant l'association d'isoniazide ou d'isoniazide et de streptomycine ont été considérés des plus satisfaisants. On considère donc que la α-éthyl-thioisonicotinamide, comme c'est le cas pour la cyclosérine et la viomycine, est un médicament présentant un grand intérêt pour le traitement des cas souvent graves de tuberculose pulmonaire chronique. Quand elle est associée à d'autres drogues, la thioamide a déterminé une amélioration des lésions et a supprimé l'infectiosité chez des malades déjà beaucoup traités antérieurement, mais qui hébergeaient des souches complètement résistantes *in vitro* à l'isoniazide et à la streptomycine.

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THE NATURAL HISTORY OF TUBERCULOSIS IN THE HUMAN BODY[1,2]

I. The Demonstrable Primary Pulmonary Infiltrate

J. ARTHUR MYERS

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Introduction

The natural history of tuberculosis has been studied in detail in several species of animals. The early course of the disease following invasion of tubercle bacilli, with particular reference to the distribution of organisms, has been observed by a number of workers.

Review of the Literature

Calmette (1), Ravenel (2), and others demonstrated that, when tubercle bacilli are introduced into the digestive tract of animals, they appear in chyle and the blood stream within a few hours, and later in the lungs. Krause (3) injected tubercle bacilli subcutaneously into inguinal regions of animals and found that they had reached bronchial lymph nodes and lungs within a few days. Lemon and Montgomery (4) introduced tubercle bacilli into the pleural spaces of rabbits and in a short time found that some had been carried to other organs, including those remotely located from the point of entrance. Regardless of the portal of entry, tubercle bacilli are promptly ingested by neutrophils and deposited in various parts of the body.

Vorwald (5) made hour-by-hour observations on the reaction of tissues to tubercle bacilli by injecting organisms into the blood stream of normal rabbits. Within a single hour, reactions occurred which represented the beginning of tubercle formations. Details of the further development of tubercles were carefully studied.

Later stages in the history of tuberculosis have been extensively studied in animals from necropsies arranged at will which have shown that the disease remains benign until the tissues become sensitized to tuberculoprotein. Thereafter, free tubercle bacilli result in specific and often intense inflammation followed by necrosis and other evidence of progressive clinical tuberculosis.

Lemon and Montgomery (4), Paterson (6), and Lemon and Feldman (7) found that significant tuberculous pleural effusion can be produced only in sensitized animals.

From 1917 through the fiscal year 1958, veterinarians of the United States did post-mortem examinations on 4,077,995 cattle slaughtered because they reacted to tuberculin (8). In this large volume of necropsies tuberculosis in all stages of its natural history was seen, from microscopic lesions to those which caused death. This has thoroughly convinced well-informed veterinarians that a characteristic tuberculin reaction practically always indicates the presence of at least microscopic tuberculous lesions and that sensitivity to tuberculoprotein is prerequisite to the development of clinical disease.

To obtain the early natural history of tuberculosis in human tissues it has been necessary to patch together lesions found at necropsy or in biopsy material usually being searched for other conditions.

After it became possible to detect the presence of sensitivity to tuberculoprotein by the tuberculin test, the specificity of the reaction could be determined only by finding lesions in the reactors. Inasmuch as early lesions are nearly always extremely small and cause mild or no significant symptom, the specificity of the test could be determined only by post-mortem studies or from biopsy material. From 1908 to 1912, Ghon (9), of St. Anne's Hospital, Vienna, made meticulous post-mortem examinations of the bodies of 184 children who during life presented no evidence of tuberculosis except a reaction to tuberculin administered by the epidermal method of Pirquet. The main object of this study was to determine whether the tuberculin reaction always indicated the presence of tuberculosis. In the 184 children who had died from other causes Ghon found this disease in all but one whose body was not completely examined. When his first examination failed, he always took a second look, and sometimes a third, before locating lesions because of their minuteness or location. In five of the bodies, the lesions were located extrathoracically. Following this, Ghon said, "From the standpoint of the pathological anatomist, my researches, which in regard to the question are not limited to the cases

[1] From the School of Public Health and Department of Medicine, University of Minnesota, Minneapolis 14, Minnesota.

[2] Prepared with the aid of the H. Longstreet Taylor Foundation, established by the Minnesota Tuberculosis and Health Association.

described here, justify me in concurring entirely with those who advocate the specific value of the tuberculin reaction."

Thus the lesions Ghon found were too small to be located by conventional physical examination or by roentgenographic inspection during life. However, their presence was made known by the tuberculin reaction.

Wallgren's classic study (10) established that the interval of time between invasions of tubercle bacilli in babies and sensitivity to tuberculoprotein detectable by the tuberculin test is from three to seven weeks. Essentially the same period has been observed in older children and adults.

With methods of examining living tuberculin reactors who are known to have at least microscopic lesions, it is possible to determine the later natural history of the disease in the lungs only when lesions become gross and are so located and have the consistency to cast visible roentgenographic shadows.

How frequently such gross conditions develop among persons who become invaded with tubercle bacilli, how soon after the invasion occurs, et cetera, can be determined only by long-term studies of a given group of persons.

Materials and Methods

From May, 1921, until November, 1941, 19,045 children from birth through high school age made 85,925 visits to one of the clinics of the Minneapolis Division of Public Health (11). The original routine procedure in this clinic consisted of history, conventional physical examination, tuberculin test (epidermal test of Pirquet for a few years, subsequently 0.1 mg. of original tuberculin intradermally), stereoscopic roentgenograms of the chest (regardless of the tuberculin reaction), gastric lavage, and sedimentation rate when indicated.

Among the children who reacted to tuberculin, many had evidence of calcific deposits in the lungs or hilum or both while, in others, the roentgenogram showed no abnormality.

In a small percentage of the patients, on first examination, shadow-casting lesions were seen resembling those produced by areas of pneumonia. In some other children who became tuberculin converters during the study, similar shadows appeared in the lungs, usually without causing significant symptoms. These shadow-casting lesions showed little evidence of change on periodic roentgenographic examination for several months. Thereafter they resolved slowly, but were still in evidence for a year or slightly longer. At that time, or months or even years later, evidence of calcific deposits was seen at the sites of the original lesions or in the hilar region, or both. Thus, these shadow-casting infiltrates proved to be the inflammatory stage of Ghon tubercle formation (12).

In the occasional recent tuberculin converter, primary infiltrates were not in evidence on roentgenograms for a few months after conversion occurred. This was thought to be due to previous lack of size and consistency to obstruct films.

Many converters did not present roentgenographic shadow-casting primary pulmonary infiltrates. However, in such cases, calcific deposits often appeared in the pulmonary parenchyma or lymph nodes, or both, months or years later.

From May, 1921, to November, 1941, 300 children were seen in the clinic with primary pulmonary infiltrates of size, density, and location to be demonstrable by the shadows they cast roentgenographically.

Pathologists have shown that primary pulmonary infiltrates practically always have hilar lymph node components. Therefore, it was thought that, when such infiltrates were in evidence on the film, there should also be shadows indicating enlargement of the lymph nodes in the hilum or paratracheal region. However, observation showed that such shadows were not in evidence in a considerable number of cases. This apparent discrepancy was explained by McPhedran (13) who, by roentgenographic inspection and postmortem examination, revealed that lymph node enlargements are undetectable on roentgenograms unless they encroach upon the lung parenchyma.

The primary infiltrates in these 300 children varied in size from those scarcely large enough to be demonstrable to big dense homogeneous areas. They were not limited to any lobe or area—some appeared even in the apices of the upper lobes.

When primary pulmonary tuberculous infiltrates were present in tuberculin reactors on first testing, there was nothing characteristic about the roentgenographic shadows to aid in differentiating them from other disease conditions or even from the reinfection type of tuberculous lesions. When other conditions (such as pneumonia) were excluded, periodic observations including roentgenographic inspection of the chest was the only way of determining with certainty whether the lesions pursued the usual slow resolution of a primary tuberculous lesion in an otherwise apparently normal individual.

The fact that children rarely develop chronic clinical reinfection pulmonary tuberculosis prior to adolescence is helpful in differentiating the type of lesion in children.

Observations

In this group of 300 children, symptoms were absent or were so mild as to be almost a diagnostic point. When symptoms were noticeable, they consisted mainly of temperature elevation ranging from 1° to 3° F. and slight malaise. Cough and expectoration rarely occurred. The erythrocyte sedimentation rate was often mod-

TABLE 1

AGE GROUPS AND AVERAGE AGES WHEN FIRST EXAMINED

Age	Girls	Boys
2 to 6 months	4	7
7 to 12 months	15	11
13 to 18 months	3	5
19 to 24 months	18	17
3 years	11	14
4 years	13	6
5 years	4	8
6 years	16	6
7 years	8	10
8 years	5	7
9 years	10	7
10 years	3	2
11 years	6	7
12 years	7	3
13 years	6	3
14 years	2	2
15 years	5	1
16 years	1	1
17 years	1	—
18 years	1	—
20 years	1	—
Total	140	117
Average ages	6 years	5 years

TABLE 2

AGE GROUPS AND TOTAL AVERAGE AGES WHEN RECENTLY TRACED

Present Age	Women	Men
years		
18	2	1
19	2	2
20	3	1
21	3	3
22	—	3
23	1	2
24	7	1
25	5	2
26	8	9
27	4	9
28	9	4
29	3	3
30	4	6
31	10	8
32	3	8
33	—	9
34	7	6
35	10	2
36	9	4
37	9	6
38	5	2
39	4	2
40	3	3
41	5	4
42	3	3
43	2	—
44	1	—
45	3	—
46	1	—
47	—	—
48	1	—
49	1	—
Average ages	32.3 years	30.9 years

erately accelerated for only a few weeks. At first when these infiltrations were seen in apparently well children, it was believed that they represented clinical tuberculous lesions. In 1935 a report was made on the treatment of such children as follows:

"When our work began, we strongly recommended sanatorium care for every child who was found to have a focus of first-infection tuberculosis in the pneumonic stage. Many families refused to carry out our recommendations because there were no outward manifestations of tuberculosis. We then advised that their children who were old enough be admitted to the Lymanhurst School. Many even refused this for the same reason and insisted upon having their children remain at home with no treatment whatever. Thus, our children were divided into three groups, which have given us an unusual opportunity to observe the effects of treatment upon the first-infection type of disease. The first group consists of those whom we sent to sanatoriums; the second, of those sent to a special school (Lymanhurst); and the third, of those who remained at home with no treatment.

"Among 155 children who had been observed from the time the disease was in the acute inflammatory stage until lime or fibrous strands were deposited in sufficient quantity to be visualized by x-ray film, 19 were treated in sanatoriums and 50 in the Lymanhurst School, while 86 remained in their homes. Of the 155 children, 136 were traced in 1935.

"Among the 136 traced, we are unable to see any difference in the course of the disease, regardless of whether the children were treated as strict bed-patients, were sent to a special school, or remained as active as any normal child is in its home. In fact, with the exception of three who developed the reinfection type of disease (to be discussed later), the entire group traced in 1935 are apparently perfectly normal, healthy individuals, going about the activities of life just as other children in their communities who have not had tuberculosis. The ultimate results in our cases have been equally good whether or not special treatment was administered. Therefore, the immediate prognosis of tuberculosis of the first-infection type among children and young adults is excellent, either in the absence or presence of treatment.

"The ultimate prognosis with regard to tuberculosis, however, cannot yet be determined for this group of children until years have elapsed, because it will depend upon the number who later develop the reinfection and destructive type of disease either from endogenous or exogenous sources."

Since this report was made in 1935 nothing has been observed among children subsequently seen with demonstrable primary pulmonary infiltrates which differed significantly from those seen before 1935.

Thus, the natural history of the Ghon tubercles had been observed from the time initial primary infiltrations were visible by roentgenographic shadows until the sharply outlined calcified stage which Ghon described was in evidence.

The observations of the present writer, beginning in 1920 on 2,266 persons who became infected with tubercle bacilli at the age of nineteen years or older and had 27,099 person-years of follow-up observation, revealed 78 with demonstrable primary pulmonary infiltrates (14).

Most of these 300 children and 78 adults would not have been known to have had tuberculosis had it not been for examinations because of history of exposure and prompt examination of recent tuberculin converters, and their primary pulmonary infiltrates would have resolved with no outward manifestation.

While the early natural history of primary tuberculosis was established among these 300 persons when they were children, an attempt has recently been made to learn of subsequent developments. Efforts to trace these persons proved difficult and time-consuming. (This investigation will be discussed in a later communication.) However, 267 (89 per cent) have been located, 10 of whom have not yet been re-examined. Twenty-six had died.

One hundred and forty of the 257 were girls. The average ages of six years for girls and five years for boys at the time of first examination are shown in table 1. The age groups of the 241 subjects still living at the time of recent tracing, with averages of 32.3 years for women and 30.9 for men, may be seen in table 2. The surviving 231 who have had recent examinations have had 6,009 person-years of follow-up observation (table 3).

The course of most demonstrable primary pulmonary infiltrates observed in these children is illustrated in figures 1 *A*, *B*, and *C*.

TABLE 3

PERIOD OF FOLLOW-UP OBSERVATION

Years	Women	Men	Total Years Followed
10	—	1	10
15	—	1	15
16	—	3	48
17	2	—	34
18	4	1	90
19	3	3	114
20	6	3	180
21	5	6	231
22	8	9	374
23	7	12	437
24	10	6	384
25	10	5	375
26	11	7	468
27	12	10	594
28	9	5	392
29	5	10	435
30	6	4	300
31	10	8	558
32	5	4	288
33	5	1	198
34	4	3	238
35	5	1	198
36	1	—	36
Grand total	128	103	6,009

TABLE 4

CAUSES OF DEATH OF 26 PATIENTS WHO SURVIVED A TOTAL OF 296 YEARS AFTER FIRST EXAMINATION

Deaths		Years of Follow-up Observation
Infections	6	69
Accidents	6	100
War	2	31
Embolism (pulmonary)	1	10
Malignancy (pulmonary)	1	34
Tumor, brain	1	22
Tuberculosis	9	
4 meningitis 1 miliary 2 pneumonia		4
2 chronic pulmonary		26
Total	26	296

Mortality

Nontuberculous conditions: Six of the 26 patients not living died from nontuberculous infections; 6, from accidents; 2 in war; and one each, from pulmonary embolism, pulmonary malignancy, and brain tumor (table 4).

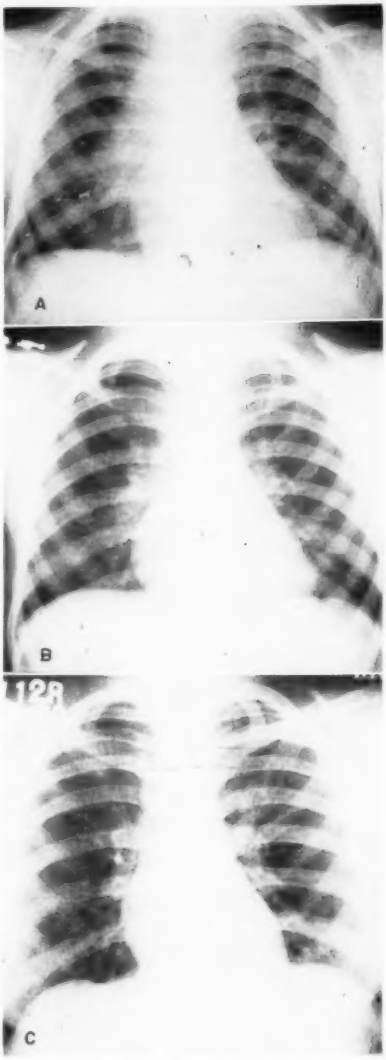


FIG. 1. *A*. A Negro female was first seen at the age of two months, on February 17, 1928. She still did not react to tuberculin through September, 1929. On March 18, 1930, she reacted strongly. All roentgenograms of her chest were clear until March 25, 1930, when an area of infiltration was seen adjacent to the right hilar region and extending into the lung parenchyma. She had no symptom of disease.

B. The lesion appeared unchanged for several months, then slowly resolved until December 17, 1931, when there was only evidence of enlargement of the hilar shadows with slight calcification.

C. By September 12, 1932, the only remaining evidence of disease was the calcification in the right hilar region. The appearance then was the same as on November 7, 1940. The patient had continued in excellent health when traced in 1958.

Acute tuberculosis: Nine deaths were attributed to tuberculosis, 7 of which were acute rapidly fatal forms (4 meningitis, 2 pneumonia, one miliary). Three of the 4 patients with meningitis died within eight months and one within twelve months after the first examination. One died at the age of one year, 2 at the age of one and a half, and the fourth at two and a half years. Apparently these 4 children had bacilli deposited in or adjacent to the central nervous system at the time of the initial invasion. From the lesions they produced, bacilli later found their way into the subarachnoid space. Prior to the work of Rich and McCordock (15) the pathogenesis of meningitis was explained on theoretic grounds. However, their experimental studies and postmortem examinations on 77 persons who died from this condition revealed that meningitis is due to endogenous reinfection from previously existing lesions in or adjacent to the central nervous system.

One child with miliary disease lived three months after the first examination in the clinic and died at the age of one year. This child obviously had a lesion located adjacent to a blood or lymph vessel when first seen and organisms from that lesion endogenously reached the blood stream.

One with tuberculous pneumonia lived six months after the first examination and died at the age of nine months. The other lived five months and died at the age of two years. The fatal disease could have resulted from direct extension of bacilli from primary pulmonary foci to allergic tissue or from tuberculous lymph nodes or other lesions eroding into bronchial ramifications from which tubercle bacilli were aspirated toward the periphery.

Chronic pulmonary tuberculosis: One child who had primary pulmonary infiltration at the age of four years died when twelve years old. He had chronic pulmonary disease and a congenital heart condition. Thus, an interval of eight years elapsed before he died from a chronic reinfection type of pulmonary tuberculosis. This is about

the age when such chronic reinfection begins to appear in previous tuberculin reactors, but death from this form of tuberculosis is unusual at this age.

A girl, who at the age of three years had a primary pulmonary infiltrate, died from chronic pulmonary tuberculosis at the age of twenty-two years. She represents the "garden variety" of evolution of chronic tuberculosis in the human body.

These 26 persons had survived a total of 296 years after the first examination (table 4). Those who died from nontuberculous conditions had survived 266 years, no evidence of clinical disease having appeared.

Morbidity

Among the 241 present survivors are 231 for whom complete information is available. The following survivors later developed evidence of clinical tuberculosis:

A one-year-old girl when first seen in October, 1936, did not react to tuberculin. Her father was a former sanatorium patient. She remained a nonreactor through November, 1937, but reacted strongly in August, 1938, when a roentgenogram revealed a sizeable area of infiltration in the base of the right lung. This did not change appreciably through March, 1939; but in March, 1940, the evidence of infiltration was gone, and at its former site was a large deposit of calcium. Periodic examinations through 1944 revealed no change. The patient was not seen in the clinic again, but in June, 1949, she entered a sanatorium with bilateral pulmonary disease with cavitation in the right lung. Pneumothorax was administered in July, 1949, phrenic nerve interruption in September, and pneumoperitoneum in November. The left lung improved promptly, and the lesions had remained stable through 1957.

In 1952 she was discharged from the sanatorium while still on pneumoperitoneum. In January, 1954, she was readmitted to the sanatorium and pneumoperitoneum was discontinued, antituberculous drugs were administered, and the lower lobe of the right lung was resected in April. She was discharged about one year later and has remained in apparent good health.

A girl one year old (1929) was examined because her father had contagious tuberculosis and a two-year-old brother had recently died from tuberculous meningitis. The patient reacted to tuberculin, and a roentgenogram of her chest in April, 1929, revealed evidence of infiltration in the upper lobe of the left lung. In June, 1929, she had massive pleural effusion from which acid-fast bacilli were recovered (figure 2*A*). After the fluid was aspirated and absorbed, the infiltrate was still demonstrable (in November, 1929). The pulmonary infiltrate gradually resolved and calcific deposits appeared in the area and in the left hilar region (figure 2*B* and *C*). No change has since been seen on periodic examination. The patient has remained well and has two children who do not react to tuberculin.

A girl of fifteen months had been exposed to an aunt who had tuberculosis but, when the child was examined in February, 1932, she did not react to tuberculin. However, in December of that year, she had "converted." A roentgenogram of her chest revealed no evidence of disease in February, 1932, but in December an infiltration was present in the right lung with enlargement of the right hilar structures. Two months later there was evidence of slight resolution, but the roentgenographic appearance of the lesion remained essentially the same until October, 1935, when there was evidence of calcification in the right hilar region. Periodic inspection of the chest showed no change through September, 1942. The patient was not seen again in the clinic, but in August, 1947, she was admitted to a sanatorium with tuberculosis of both kidneys and the urinary bladder. During her twenty-eight months of residence at the institution, one kidney was removed. She was readmitted in 1950 for a period of seven months. After discharge, she continued on antituberculous drugs for the next two years. When last traced, she was apparently well.

A boy of two years was first seen in July, 1933, because his father had tuberculosis. He reacted to tuberculin, and a primary infiltrate was in evidence in the right lung with enlargement of the hilar structures. The condition slowly resolved, and in May, 1935, there was evidence of calcific deposits. No change had occurred in October of that year. He did not return to the clinic, but in 1944 he developed clinical disease for which he was treated for approximately a year. Since then he has remained well.

A girl of three years was first seen on November 1, 1923, because of exposure to her tuberculous mother. She reacted to tuberculin, and infiltration was demonstrable in the base of the right lung. She did not return to the clinic, but was in a sanatorium from July to December, 1924, because of an abnormal roentgenographic shadow. In January, 1950, she was admitted to a hospital in another state for bronchial tuberculosis which was treated successfully by bed rest and antituberculous drugs. She has since remained in good health.

A boy of five years was first seen on June 5, 1922, because his mother had tuberculosis. He reacted to tuberculin and an infiltrate at the base of the right lung cast a homogeneous shadow on the roentgenogram. He remained in good health until September, 1949, when he was admitted to a sanatorium with moderately advanced pulmonary tuberculosis. Sputum cultures revealed acid-fast bacilli. While on bed rest, evidence of cavitation appeared in the area of disease in the upper lobe of the left

lung. Antituberculous drugs were administered. Adhesions prevented satisfactory artificial pneumothorax. An upper five-rib thoracoplasty was done in April, 1950. The boy has been well since discharge in January, 1951.

A girl of nine years was first examined in November, 1935, having been exposed to a tuberculous uncle. She reacted to tuberculin, and an infiltrative process was present at the level of the first rib and the first interspace on the right side. In March and April, 1936, there was some resolution of the lesion. By November there was evidence of calcific deposits within the area of the infiltrate. The condition apparently remained unchanged until May, 1940, when there was an increase in the extent of disease at the site of the primary focus. The patient was not seen again in the clinic, but was admitted to a sanatorium in September, 1940, where artificial pneumothorax was instituted immediately on the right side, and one year later on the left side. She was discharged from the sanatorium in December, 1944, but continued under artificial pneumothorax until June, 1954, when antituberculous drugs were prescribed and administered for the next two years. She has since been apparently well.

This girl had been under the close care of a pediatrician since infancy. Periodic tuberculin tests and roentgenograms of her chest were negative. When she was nine years old, a maid was employed without a pre-employment examination. Although the maid had cough and expectoration, several weeks elapsed before she was examined. She had advanced pulmonary tuberculosis in the infectious state. Re-testing of the child then resulted in a strong reaction. A roentgenogram (1935) revealed the shadow-casting lesion in the left lung (figure 3*A*).

There was no symptom of disease, but her pediatrician kept her on strict bed rest in the home, with nursing care for one year. There was slow resolution with residual calcific deposits at the level of the left second interspace and in the hilar region. These lesions represented a primary tuberculosis complex.

The patient was in excellent health, and subsequent roentgenograms of her chest showed no change until the summer of 1942, when a periodic film revealed evidence of disease in the right lung (figure 3*B*). Symptoms appeared and tubercle bacilli were recovered from gastric washings. This was reinfection clinical tuberculosis which was treated successfully. Thus, under medical observation, this girl developed primary tuberculosis with demonstrable pulmonary infiltrates which receded in the usual manner. However, at the age of sixteen years, a chronic reinfection type of disease appeared.

A girl of thirteen years was first seen on September 16, 1922, when she reacted to tuberculin, and a small area of parenchymal infiltration extending out from the right hilum cast a roentgenographic shadow. On January 30, 1923, evidence of calcific deposits was seen in the right hilar region and what

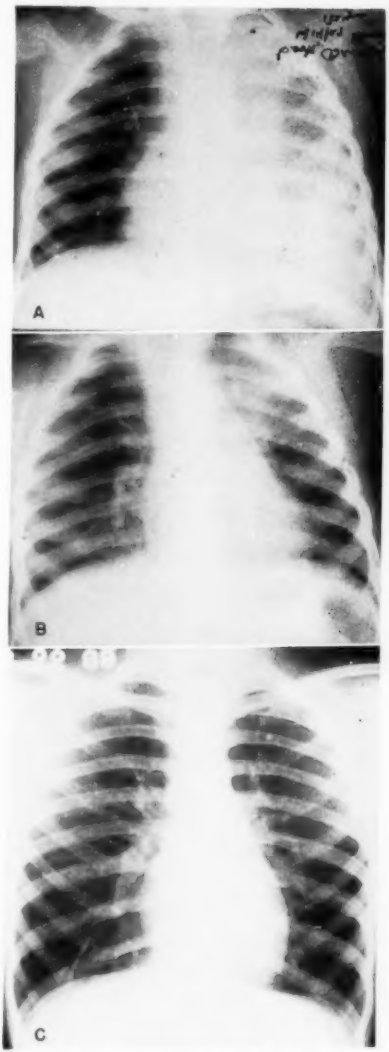
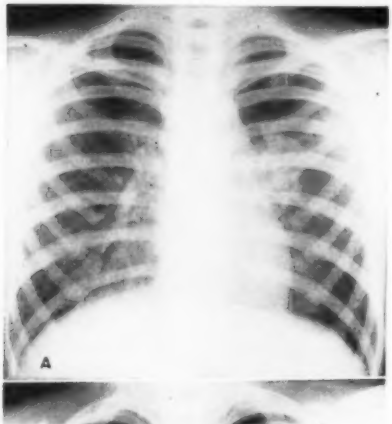


FIG. 2. *A*. Roentgenogram taken on June 14, 1929, reveals evidence of pleural fluid on the left side of this one-year-old girl.

B. Film of same chest (November 16, 1929) after fluid disappeared shows primary infiltrate in the upper lobe of the left lung which appears essentially the same as it had in April, 1929, before fluid appeared.

C. Film of same chest (June 22, 1939). Note evidence of calcific deposits at level of the left first rib anteriorly and in left hilar region. There had been no change in the appearance of roentgenograms of the patient's chest through August, 1956. Clinically she has remained entirely well.

appeared to be a fibrous infiltration extended into the apex of the right lung. No change was apparent on a chest film obtained in June, 1928. The patient remained in good health until 1948 and 1949 when



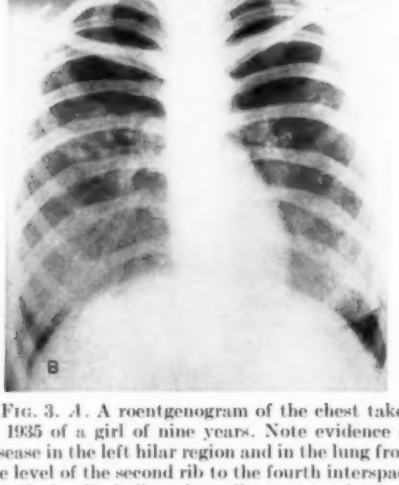


FIG. 3. *A*. A roentgenogram of the chest taken in 1935 of a girl of nine years. Note evidence of disease in the left hilar region and in the lung from the level of the second rib to the fourth interspace anteriorly. Periodic tuberculin tests and roentgenograms of her chest over several years had been negative until 1935 when she was exposed to a person with contagious tuberculosis. No symptom was present, but her pediatrician insisted upon strict bed rest for approximately a year. Resolution of the primary infiltrate slowly occurred and periodic roentgenograms then revealed calcific deposits in the left lung at the level of the second interspace and in the left hilar region.

There was no change until December, 1942, when evidence of clinical reinfection tuberculosis appeared in the right lung at the level of the second and third interspaces anteriorly. Tubercle bacilli were recovered. (*See* text.)

bilateral renal tuberculosis was diagnosed. Treatment included bed rest and antituberculous drugs. She has since been apparently well.

A girl of nine years did not react to tuberculin. However, when she was thirteen years old she reacted and had a demonstrable area of infiltration at the level of the second interspace anteriorly on the left side in January, 1929. No evidence of clinical disease developed through November, 1936. When her fourth and fifth children (twins) were born, there was a flare-up of the shadow-casting lesion in the left upper lung field which subsided in about six months. The patient has since been apparently well.

A girl of ten months was first seen on February 15, 1934, after exposure to an adult sister with sputum positive for tubercle bacilli. She reacted to tuberculin. A roentgenogram revealed an area of infiltration in the upper lobe of the left lung. Throughout both lungs there were numerous areas of increased density strongly suggestive of miliary tuberculosis. Late in July the disseminated shadows appeared slightly smaller. No change was then seen until November, when further clearing was in evidence.

In April, 1935, the primary infiltrate remained essentially the same as on the initial examination, but the shadow-casting lesions throughout both lungs had disappeared.

The primary infiltrate slowly resolved in the usual manner and deposits of calcium appeared.

From the time the patient was first seen at the age of ten months until all shadows had disappeared and calcification occurred in the primary focus, 48 roentgenograms of her chest were studied. She had no symptom at any time and has remained well with no apparent change in roentgenographic findings through the present time. Aside from the primary pulmonary tuberculous infiltrate, an accurate diagnosis was not made. The widely disseminated lesions throughout both lungs could have been due to tubercle bacilli focalized before allergy was established and the lesions thus behaved as primary infiltrates. However there has been no evidence that they became calcified.

Among the 231 traced survivors who have been examined recently, 9 subsequently developed clinical tuberculosis, leaving 222 who apparently have remained free of this form of disease. In addition to the 9 who had died from tuberculosis among the 257 traced, a total of 18 had developed clinical tuberculosis during the 6,305 person-years followed.

No conclusion can be drawn with regard to the ultimate appearance of reactivations of old lesions and new demonstrable areas of disease among the 231 survivors. The women are now on an average about thirty-two and the men

thirty-one years old. The average length of life in this country now closely approaches seventy years. Therefore, these persons will not attain the half-way mark for approximately four or five years. Morbidity and mortality from tuberculosis in this area now are highest after the age of forty years. Therefore much time remains for clinical lesions to evolve.

Discussion.

A study such as this, which was begun in 1921, could not now be initiated in this area because the number of cases of this kind which develop is too small. On the basis of sample tuberculosis testing here, reactors among grade school children have decreased from 70 per cent in 1916, 47 per cent in 1926, 19 per cent in 1936, 8 per cent in 1944, to 4 per cent in 1954 (16). Inasmuch as each tuberculin reactor is a potential case of clinical tuberculosis, morbidity and mortality rates run parallel with the incidence of infection (table 5). In 1926, when 47 per cent of grade school children (population about 400,000) reacted to tuberculin, 150 persons less than twenty years of age were reported to have clinical tuberculosis and 29 died, 15 of whom had meningitis. Obviously, the incidence of infection was high in persons more than twenty years of age, and the number of clinical cases among them brought the total to 1,198, with 230 deaths among persons of all ages.

By 1956 in the same city (population about 550,000), only 17 cases (of which only 5 were clinically significant) were reported in patients less than twenty years old, and no one less than thirty-five years old died (table 5). There were no cases of tuberculous meningitis, pneumonia, or miliary disease.

During the interval of thirty years, when the incidence of tuberculin reactors among grade school children decreased to 4 per cent, obviously the lowered incidence of reactors extended from childhood into several decades of life. Thus, morbidity and mortality among persons of these age groups decreased accordingly. In 1956, only 456 persons of all ages were reported to have clinical tuberculosis and only 32 died (thirty-five years of age or older).

It is doubtful whether such a study could now be conducted anywhere in the world inasmuch as antituberculous drugs are so often administered as soon as the disease is found by the tuberculin test, and demonstrable primary infiltrates are frequently even resected.

TABLE 5

Minneapolis Tuberculosis Cases and Death Rates per 100,000 Population 1926 and 1956

Year	Cases				Deaths			
	Less than 20 Years		All Ages		Less than 20 Years		All Ages	
	Number	Rate	Number	Rate	Number	Rate	Number	Rate
1926	150	108.8	1,198	276.2	29*	21.0	230	53.0
1956	17*	11.5	456	84.3	0	0.0	32	5.9

* Courtesy F. G. Gunlaugson, M.D., Director Bureau of Preventable Diseases, Minneapolis Division of Public Health.

It might be argued that knowledge of the natural history of tuberculosis is no longer important or necessary because of the present antituberculous drugs and resectional surgery. Such a statement may be countered with the fact that present drugs do not kill, and resectional surgery does not remove, all tubercle bacilli. It is not known how long present drugs may be of value because of mutation, et cetera, of tubercle bacilli or whether other equally good or better drugs are forthcoming. There is no hope that surgery will remove all tubercle bacilli since, with the initial invasion, foci are established in various parts of the body. It would not be possible to find them all during life. Moreover, as yet there is little evidence that present drugs have much, if any, effect on the course of demonstrable primary pulmonary infiltrates. Therefore, after the drugs are administered and even when the one demonstrable primary focus is removed, the individual still harbors foci of living tubercle bacilli.

The 300 children who have been observed must not be compared with groups of children reported from hospitals and sanatoriums, for, in the last-named circumstances, the children were admitted because of illness due to complications including bronchial involvement, atelectasis resulting from accumulations in bronchi or extrinsic pressure from enlarged lymph nodes, or acute and chronic reinfection forms of tuberculosis, et cetera.

Although such hospitalized children require treatment for the complications and reinfections, apparently they constitute an exceedingly small

percentage of all children who have primary tuberculosis. On the other hand, children who are found with demonstrable, primary pulmonary infiltrates constitute a small percentage of those who have primary tuberculosis, but whose lesions do not have size, consistency, or location demonstrable roentgenographically.

Probably the main reason that the majority of primary lesions are benign is that their development begins in nonsensitized tissues whose reactions to tubercle bacilli are not specific, and thus they do not differ significantly from those caused by such foreign material as particles of silicon dioxide. The defense mechanism works without interference and the tubercle bacilli are well surrounded by the time the tissues become sensitized to tuberculoprotein. On the other hand, tubercle bacilli lodged in sensitized tissues evoke an intense specific reaction because tuberculoprotein is a violent poison to such tissues. Hence, necrosis is likely to occur as a part of progressive clinical disease. Although many types of reinfection of tuberculous lesions come under control spontaneously, there is a strong tendency for them to progress intermittently or continuously. Thus, every such lesion deserves prompt treatment.

In fact, demonstrable primary pulmonary infiltrates behave as differently from the reinfection type of lesions as though they were caused by different etiologic agents. Therefore, it is exceedingly important to differentiate in diagnosis between primary pulmonary infiltrates and those of the reinfection type. Indeed, this is as important as differentiation between tuberculosis and other pulmonary lesions, such as histoplasmosis.

From the present writer's observations of demonstrable primary infiltrates, it has long been emphasized that no study of tuberculosis in any aspect can be considered well documented unless meticulous care is exercised to differentiate between primary and reinfection types of lesion.

Wherever such differentiation is not practiced, erroneous deductions are made with reference to efficacy of therapeutic and preventive measures. For example, various methods of treatment may be credited with beneficial effects when in reality they are valueless because the lesions under consideration are benign primary infiltrates which nearly always recede, regardless of how much or how little treatment is administered. In fact, some forms of treatment might have been considered responsible for the course the primary pulmonary infiltrate took in the present group of 300 children if the natural history of the disease had not been observed. Again, without proper differentiation, primary infiltrates may be grouped with the reinfection type of lesion, so it appears that more control subjects develop clinical disease than those who have primary lesions produced in the skin by living tubercle bacilli, i.e., BCG, with the view to prevent more serious tuberculosis.

Among the 300 children with demonstrable, primary pulmonary infiltrates, 7 of the 267 recently traced had died from acute disease, including meningitis, pneumonia, and miliary dissemination. The last death in this group occurred in 1940. These 9 deaths provide information concerning the natural history of tuberculosis in persons who have uncomplicated demonstrable primary pulmonary infiltrates. The pulmonary lesions played no apparent role in the fatal conditions.

Nine among the 231 recently traced survivors had developed the reinfection, clinical type of tuberculosis. Here again, the natural history is seen in such a group of cases, and as yet no method of preventing such developments has been devised.

While mortality and morbidity to date may seem small, they may still be considerably increased as the subjects under consideration have not attained the halfway mark in the span of life which now obtains in this country. Even these mortality and morbidity rates to date represent a tremendous waste if extended to the millions of persons in the world with primary pulmonary tuberculous infiltrates.

Thus, while the demonstrable primary pulmonary infiltrate is a benign condition which must not be classified with clinical lesions, its host, like all tuberculin reactors, harbors other foci of tubercle bacilli. Consequently, the host should be kept under close surveillance for later evolution of clinical lesions, since the benignity of the primary pulmonary infiltrate is no assurance against the development of such lesions. Thus the importance of protecting all persons against infection with tubercle bacilli is emphasized.

Summary

From May, 1921, to November, 1941, 300 children were examined who had demonstrable

primary pulmonary tuberculous infiltrates. After a few months, the infiltrates began to slowly resolve and within approximately a year had disappeared from roentgenographic view except for evidence of calcific deposits at their former sites, in the regional lymph nodes, or both. Thus, a phase of the natural history of tuberculosis was observed as primary pulmonary infiltrates were seen to represent the inflammatory stage of Ghon tubercle formation.

This study shows that demonstrable primary pulmonary infiltrates behave as differently from the reinfection type of lesions which produce similar roentgenographic shadows as though they were caused by different etiologic agents.

In diagnosis it is exceedingly important to differentiate between such primary infiltrates and those of the reinfection type of tuberculosis. Whenever such differentiation is not made, erroneous deductions are drawn with respect to efficacy of therapeutic and preventive measures.

Recently 267 of the 300 children were traced, but 10 were not examined. Twenty-six had died. Nine of the deaths were due to tuberculosis, 7 of which were acute, rapidly fatal reinfection forms (meningitis, pneumonia, and miliary dissemination). Two other patients died from chronic pulmonary tuberculosis, eight and nineteen years, respectively, after the primary pulmonary infiltrates were first found.

Among the 231 survivors traced and examined, 9 had subsequently developed reinfection chronic clinical tuberculosis, only one in the area of the old primary infiltrate.

The remaining 222 have developed no evidence of clinical disease. However, no final conclusion can be drawn because these patients are still less than half-way through the natural span of life.

The benign character of the demonstrable primary pulmonary infiltrate is no assurance against the later development of both pulmonary and extrathoracic clinical disease. Thus the importance of protecting all persons from infection with tubercle bacilli is emphasized.

Sumario

La Historia Natural de la Tuberculosis en el Organismo Humano. I. El Infiltrado Pulmonar Primario Descubrible

De mayo de 1921 a noviembre de 1941, se examinó a 300 niños que tenían infiltrados tuberculosos primarios descubribles en el pulmón. Al cabo de algunos meses, los infiltrados comenzaron a resolverse y aproximadamente en término de un año habían desaparecido de la escena radiográfica, excepto por signos de depósitos calcíficos en los sitios anteriores, en los ganglios linfáticos regionales o en ambos. Se observó, pues, una fase de la historia natural de la tuberculosis al contemplar infiltrados pulmonares primarios que representaban la etapa inflamatoria de la formación del tubérculo de Ghon.

Revela este estudio que los infiltrados pulmonares primarios descubribles se comportan tan distintamente de las lesiones de tipo de reinfección que producen sombras roentgenográficas semejantes como si fueran ocasionados por distintos agentes etiológicos.

En el diagnóstico es sumamente importante diferenciar entre dichos infiltrados primarios y los de la tuberculosis de tipo de reinfección. Siempre que no se hace tal diferenciación, se sacan deducciones erróneas con respecto a la eficacia de las medidas terapéuticas y profilácticas.

Hace poco se descubrió el paradero de 267 de los 300 niños, pero no se examinó a 10 de ellos. Veintiséis habían fallecido. Nueve de las muertes se debían a tuberculosis, siendo 7 de ellas causadas por formas agudas y rápidamente letales de reinfección (meningitis, neumonía y granulia). Otros 2 enfermos fallecieron de tuberculosis pulmonar crónica, a los ocho y diecinueve años, respectivamente, de haberse descubierto los infiltrados pulmonares primarios.

Entre los 231 sobrevivientes descubiertos y examinados, 9 habían manifestado después tuberculosis clínica crónica de reinfección, pero solamente uno en la zona del antiguo infiltrado primario.

Los 222 restantes no han manifestado signos de afección clínica. Sin embargo, no cabe sacar ninguna conclusión definitiva por encontrarse estos sujetos todavía a menos de la mitad del camino comprendido en la duración natural de la vida.

La benignidad del infiltrado pulmonar primario descubrible no garantiza contra la aparición subsiguiente de enfermedad clínica tanto pulmonar como extratorácica. Recalca, pues, esto la importancia de resguardar a todas las personas contra las infecciones por bacilos tuberculosos.

Resume

L'évolution naturelle de la tuberculose dans le corps humain. I. L'infiltrat pulmonaire primaire démontrable

Du mois de mai 1921 à novembre 1941, 300 enfants porteurs d'infiltrats pulmonaires tuber-

culeux primaires démontrables ont été examinés. Au bout de quelques mois, les infiltrats ont commencé à se résorber lentement et, dans l'espace d'environ un an, ils avaient disparu des clichés; il ne subsistait que des dépôts calcifiés à leur siège initial ou dans les ganglions lymphatiques régionaux, ou en ces deux points. C'est ainsi que l'on a observé une phase de l'évolution naturelle de la tuberculose; car on considérait les infiltrats pulmonaires primaires comme représentant la phase inflammatoire de la formation du tubercule de Ghon. Cette étude révèle que l'évolution des infiltrats pulmonaires primaires démontrables est aussi différente de celle de la lésion de reinfection, dont l'aspect radiologique est semblable, que si elles étaient causées par des agents étiologiques différents.

En diagnostic, il est extrêmement important de différencier ces infiltrats primaires de ceux du type réinfection tuberculeuse. Lorsque cette différentiation n'est pas faite, les déductions qu'on peut en tirer sont erronées en ce qui concerne l'efficacité des mesures curatives et préventives.

On a pu récemment retrouver 267 enfants sur 300; 10 n'ont pas été examinés; 26 d'entre eux étaient morts; 9 des décès étaient dûs à la tuberculose. Parmi ceux-ci, 7 avaient présenté une forme aiguë de réinfection rapidement mortelle (méningite, pneumonie, dissémination miliaire); 2 autres malades moururent de tuberculose pulmonaire chronique, respectivement 8 et 19 ans, après la découverte des infiltrats pulmonaires primaires.

Parmi les 231 survivants retrouvés et examinés, 9 ont ultérieurement présenté une forme chronique de tuberculose clinique de réinfection; dans un cas seulement la lésion était localisée au siège de l'ancien infiltrat primaire.

Les 222 autres patients ne présentaient aucun signe clinique de tuberculose. Aucune conclusion définitive ne peut néanmoins être tirée, car ces malades ne sont pas encore à mi-chemin du cours normal de la vie humaine.

La bénignité de l'infiltrat pulmonaire primaire apparent n'est pas une assurance contre le développement clinique ultérieur de la maladie pulmonaire ou extrathoracique ou des deux. L'importance de protéger tous les individus contre l'infection par le bacille tuberculeux est ainsi mise en évidence.

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LATE DISCHARGE OF TUBERCLE BACILLI IN PRIMARY TUBERCULOSIS[1, 2]

EDITH M. LINCOLN AND AMPARO C. ADIAO

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INTRODUCTION

The possibility that adults who have made a clinical recovery from pulmonary tuberculosis may still occasionally discharge tubercle bacilli has been recognized for many years. The fact that tubercle bacilli may sometimes be cultured from the gastric contents of children long after the onset of primary tuberculosis is perhaps not as well known, but has been the subject of a number of excellent discussions.

The present study was undertaken to review the pediatric literature as well as the experience of the Children's Medical Service of Bellevue Hospital in order to learn, if possible, under what circumstances children discharge tubercle bacilli for a longer time than usual after primary tuberculosis. In addition, an effort has been made to explore the significance of late excretion of bacilli in terms of prognosis for the individual child. An estimate of prognosis based on long-term supervision of patients is important in deciding on the necessity for specific antimicrobial therapy in persons from whom tubercle bacilli are cultured late in the course of primary tuberculosis.

Review of the Literature

Poulsen and Andersen (1), in 1934, reported a group of 622 tuberculous children in hospitals, 199 of whom demonstrated tubercle bacilli by culture of gastric contents, an incidence of 32 per cent. Gastric lavages were continued at one- to two-month intervals until two successive cultures were negative. Twenty-six children had cultures positive for tubercle bacilli for more than six months; and 5, for more than a year. Lester (2), reporting on the same group of patients, compared the results with those obtained from older patients and concluded that tuberculous children less than ten years of age "secrete by no means as many tubercle bacilli as older children or adults." Levin (3), in 1935, studied 434 tuberculous children less than sixteen years of age, in 45 per cent of whom tubercle bacilli were demonstrated. He noted a higher percentage of cultures positive for tubercle bacilli in the younger children and also a great variation in the duration of bacillary excretion, but found that in most cases cultures became negative within a year. However, Levin observed 54 children with regressing lesions, some showing evidence of calcification, in all of whom he was able to culture tubercle bacilli from gastric washings for periods of one to four years; 4 patients had positive cultures for more than two years. Rosencrantz and Hurwitz (4), in 1940, reported 169 patients admitted consecutively to a children's tuberculosis service, in 45 per cent of whom tubercle bacilli were demonstrated by guinea pig inoculation of gastric lavages. Fifteen children were reported in whom tubercle bacilli were recovered more than six months after the initial diagnosis—in only 2 of these patients as long as two years later. These studies were all done before the era of specific antimicrobial therapy.

Dijkstra (5) studied 106 patients with primary pulmonary tuberculosis between 1948 and 1951, half of whom were less than fifteen years old; the rest were adolescents and young adults, fifteen to twenty-five years of age; 8.5 per cent continued to excrete bacilli after the lung process had become clinically inactive. The number of excreted bacilli varied a great deal. In general, fewer bacilli were cultured from patients with intermittent discharge than from patients with continuously infectious sputum. Apparently none of these patients received specific antimicrobial therapy, the only "active" treatments cited being artificial pneumothorax and lobectomy.

Vigneron (6) and Bertoye, Depierre, and Romier (7), in 1953 and 1954, reported 44 children with primary pulmonary tuberculosis who had excreted tubercle bacilli in the first months of their disease. In 10 of these children, cultures positive for tubercle bacilli could still be obtained after a year, and in one child after two years. These writers emphasize their belief that it is unlikely that this high proportion of children with late cultures positive for tubercle bacilli would be found in the general population of

[1] From the Chest Clinic of the Children's Medical Service, Bellevue Hospital; and the Department of Pediatrics, New York University College of Medicine, New York, New York.

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children with primary tuberculosis. There was no striking difference in roentgenographic or clinical pictures between the group in which tubercle bacilli persisted in the gastric contents for more than a year and the 34 patients whose gastric contents became negative within a year. Roentgenographic evidence of segmental or lobar shadows and associated bronchial fistulas were seen in both groups. Some of the children showing tubercle bacilli in gastric lavages after a year had roentgenographic evidence of calcification in the primary complex. None of these children apparently received isoniazid therapy.

Present Investigation

The records of all tuberculous children admitted to the tuberculosis ward of the Children's Medical Service of Bellevue Hospital between March, 1947, and July 1, 1956, have been studied in order to determine the number of children with primary tuberculosis from whom tubercle bacilli were cultured six months or more after the initial diagnosis of tuberculosis. Every child had three gastric lavages as soon after admission as possible and at least once monthly during the duration of the hospital stay. A large number of the patients had one or more endoscopic examinations, and the secretions obtained were always cultured. Each culture positive for tubercle bacilli was studied for drug susceptibilities. All bacteriologic investigations were done in a laboratory serving only the Children's Chest Service by technicians specially trained in tuberculosis work.

Eight hundred and forty children were studied. Of these, 309 (36.8 per cent) showed tubercle bacilli on culture of gastric lavages or bronchial secretions. Forty-nine of the tuberculous children under study were found to have chronic pulmonary tuberculosis and have been excluded from this report. Likewise, 7 children who had almost continuously positive cultures for more than six months after admission have been excluded (2 of these had miliary tuberculosis; 4 had locally progressive cavitary primary tuberculosis, and one had both of these complications). These 7 children present a special problem outside the scope of this report.

With the exclusion of the above patients, there were 253 patients with primary tuberculosis from whom tubercle bacilli were cultured at some time during their hospital stay; 40 of them (15.1 per cent) had cultures positive for tubercle bacilli six months or more after the initial diagnosis of primary tuberculosis. In 31 of these patients the cultures had reverted to positive after becoming negative; in 8 patients all previous cultures had been negative; in one patient with a complicating skeletal tuberculosis initial gastric lavages were apparently omitted. In addition to these 40 children, 4 patients will be presented who were originally observed between 1930 and 1936 and in whom tubercle bacilli were found fourteen or more years later during the period 1950–1956. These 44 patients will be considered in four groups.

Observations

Group I consists of 24 of the 44 patients. In these 24, tubercle bacilli were grown from gastric or bronchial fluids six months or more, but less than a year, after the diagnosis of primary tuberculosis; 18 of the 24 had bronchial involvement proved by endoscopic examination. The initial diagnosis in 2 children was locally progressive primary tuberculosis with cavitation and bronchogenic spread, and miliary tuberculosis was present in another patient. Only 2 children had negative chest roentgenograms: one with extrapulmonary primary tuberculosis and one who had tuberculosis of the tonsils. The most striking finding in these 24 children was the relatively large number of instances of tuberculosis of the tonsils or adenoids, or both; this complication occurred six times in the 24 patients. In addition, another child had a tuberculous uveitis, so that more than a quarter of these patients had proved active tuberculous disease draining into the upper respiratory tract, a far greater proportion of such complications than is usually seen in children with primary tuberculosis.

The appearance of tubercle bacilli six months or more after the initial diagnosis of tuberculosis could be correlated with clinical changes in only 2 patients, both of whom developed tuberculous complications draining into the upper respiratory tract. In the remaining 22 children, there was no evidence of clinical or roentgenographic worsening which could be related to the late appearance of tubercle bacilli in cultures.

Group II consists of 8 infants in whom tubercle bacilli were found one to two years after the initial diagnosis of primary tuberculosis. Three children had roentgenographic evidence of cavity within the primary focus and bronchogenic spread, 2 had had miliary tuberculosis, and again there were 2 patients with tuberculous complications of the upper respiratory tract, tuberculosis of the adenoids, and tuberculous otitis media. As in the previous group, it was not possible to relate the late cultures positive for tubercle bacilli to clinical or roentgenographic changes in the patient.

In *Group III* there are 8 children in whom cultures of tubercle bacilli were grown from fluid obtained by gastric lavage two through six

years after the initial diagnosis of primary tuberculosis. In one of these children, *T. B.*, hemoptysis preceded the positive culture, which was obtained on readmission to the hospital five years after primary tuberculosis. A two-month course of antimicrobial therapy with streptomycin, considered adequate at the time, was followed by persistently negative cultures. Resection was performed a year after readmission and showed extensive caseation in the bronchiectatic upper lobe. A culture positive for tubercle bacilli was obtained from the specimen. This is the only instance in which the reappearance of tubercle bacilli long after the initial diagnosis of primary tuberculosis coincided with clinical evidence of relapse. In patient *M. Sa.* a residual large area of calcification within the segment, which was originally obstructed, was already considered an indication for resection, and three late cultures positive for tubercle bacilli more than six years after the original diagnosis of primary tuberculosis strengthened the decision for surgery. *L. K.* originally had cavitating primary tuberculosis which was treated with streptomycin for three months, with excellent immediate results; but relapse occurred a year later with reappearance of the cavity and positive cultures. After retreatment the patient remained well, but three years later, without any clinical or roentgenographic changes, tubercle bacilli were cultured on one occasion from a gastric lavage. The patient finally underwent resection seven years after his first admission after numerous cultures had been negative for tubercle bacilli. The resected lobe showed bronchiectasis but no evidence of tuberculosis, and cultures were negative. In patients *C. R.* and *D. B.* the tissues presented similar normal histologic findings when resection was done some months after a single positive culture followed by negative cultures of gastric fluids.

J. Ba. originally presented evidence of primary tuberculosis with obstruction of the right middle lobe (figure 1*a*). Six years after her initial disease she was readmitted because tubercle bacilli were isolated from the gastric fluid three weeks after a typical lobar pneumonia, although several cultures of gastric lavage taken early in the course of the pneumonia had been negative. On readmission, the patient was entirely free from symptoms and presented roentgenographic evidence of a contracted right middle lobe and calcification in the hilar and paratracheal nodes (figure 1*b*, *c*). Numerous cultures of gastric lavages were negative for tubercle bacilli. Bronchoscopy was normal. A bronchogram (figure 1*d*) showed the crowded middle lobe bronchi which would be expected in a contracted lobe and calcification in the nodes probably responsible for the tuberculous endobronchitis in the middle lobe and anterior segment of the right upper lobe.

In the remaining 2 patients in *Group III*, tubercle bacilli were found when the children were readmitted to the hospital for re-evaluation and for possible resection of contracted lobes, sequelae of endobronchial tuberculosis. In addition, one of these children had proved tuberculous otitis and mastoiditis and continued to have intermittent discharge from the involved ear but cultures consistently negative for tubercle bacilli from these secretions.

Thus in a period of nine years and four months, 40 of 253 children with primary tuberculosis (15 per cent) had cultures of tubercle bacilli from gastric lavages more than six months after the initial diagnosis. Sixteen (6 per cent) of these children had cultures positive for tubercle bacilli more than a year later, and 8 (3 per cent) more than two years after primary tuberculosis was first recognized.

Finally, *Group IV* consists of 4 patients from whom cultures of tubercle bacilli were obtained by gastric lavage fourteen to almost twenty-six years after they were first known to have primary tuberculosis. They illustrate the type of problem which is not infrequently presented to the clinician dealing with adults who has to make decisions about the necessity of medical or surgical therapy without the help of documentation regarding the past history of the patient. All of these patients had residual contracted upper lobes secondary to tuberculous endobronchitis and gave evidence of tubercle bacilli on culture of gastric lavages obtained as part of evaluation or follow-up investigations.

E. C. had been known to have primary tuberculosis in 1936, when at the age of two years she had roentgenographic evidence of obstruction of the left upper lobe and a positive tuberculin test. More than ten years later during adolescence she was readmitted to Bellevue Hospital for study, with the possibility of a diagnosis of active pulmonary tuberculosis. A roentgenogram showed

contraction and calcification of the left upper lobe. Repeated gastric lavages were negative for tubercle bacilli, but the patient was sent to a sanatorium where further cultures were also negative. However, three years later, on a routine follow-up examination, one culture of gastric contents showed tubercle bacilli; this was followed by three negative examinations. No specific treatment was given. The patient has continued well, had a normal pregnancy in 1955, and 6 gastric lavages after delivery were negative for tubercle bacilli. This patient then had a single

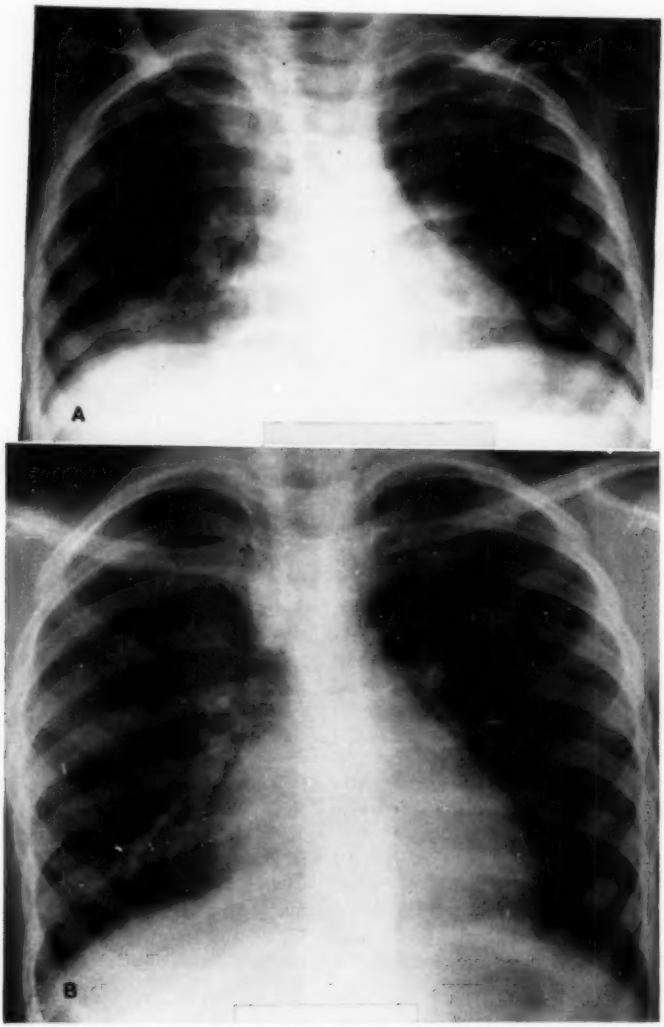

FIG. 1 (*J. Ba*). *A* (October 14, 1949). Widening of mediastinum to the right, enlarged right root shadow; clouding and streaking adjacent to the right heart shadow and above the diaphragm, interpreted as primary tuberculosis of the right middle lobe with probable bronchial obstruction.

B (February 23, 1956). Large calcified area in right paratracheal region; calcification in right root; heavy strand extending downward from the right root containing calcifications.

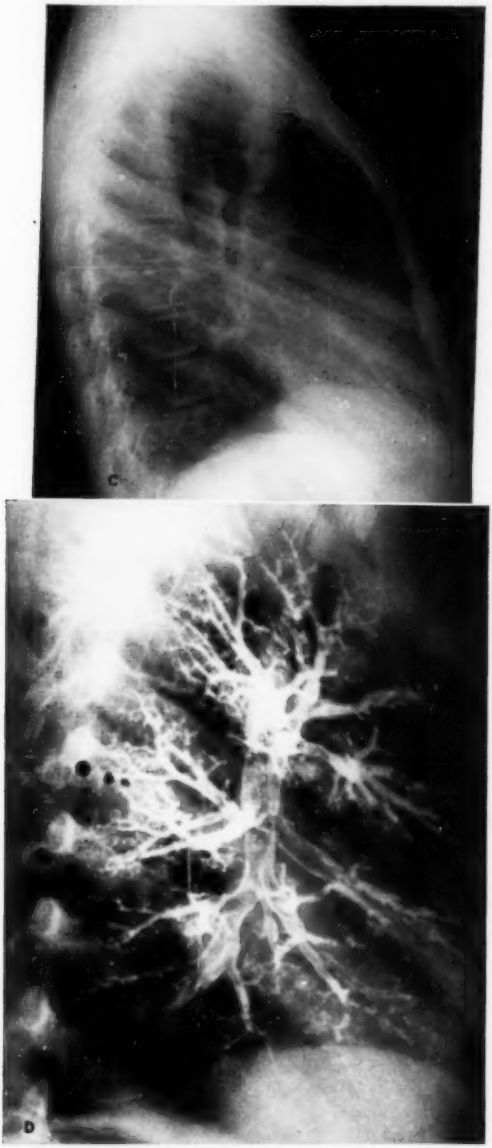

FIG. 1 (*J. Ba*). *C*. Lateral view, February 23, 1956. Large calcifying node anterior to trachea and heavy strand interpreted as contracted right middle lobe.

D. Bronchogram, April 10, 1958. Crowding of middle lobe bronchi and calcified nodes, probably responsible for endobronchitis in right middle lobe and right upper lobe.

culture of tubercle bacilli thirteen and a half years after the diagnosis of primary tuberculosis and has remained well with no subsequent positive cultures and no roentgenographic changes for eight years following the finding of tubercle bacilli.

A. L. had been seen originally for an obstructed left upper lobe and healing primary tuberculosis at the age of ten years and on frequent later follow-up examinations had shown progressive shrinking of the left upper lobe. As part of a routine follow-up examination at the age of twenty-four tubercle bacilli were found in a sputum concentrate. Bronchoscopy revealed a minute area of granulation tissue in the left main bronchus. Culture of bronchial aspiration and subsequent gastric lavages was negative for tubercle bacilli. Both surgery and antimicrobial therapy were considered, but no treatment was given. The patient remains well, with culture of gastric lavage persistently negative for tubercle bacilli and no change roentgenographically five years later.

M. G. had been known to the Chest Clinic of the Children's Medical Service from the age of four years when she had a calcifying primary tuberculosis and cultures of gastric lavages negative for tubercle bacilli. Five years later she was readmitted for a tonsillectomy and adenoidectomy, and again cultures of gastric lavages were negative. She was followed in her local chest clinic with periodic check-up and sputum examinations until the age of twenty-five years when, in preparation for discharge from follow-up study by the Children's Chest Clinic of Bellevue Hospital, a bronchogram was requested. Before admission to a local sanatorium for this procedure, gastric washings were examined and one culture showed growth of tubercle bacilli. This one positive result was followed by several negative cultures. However, the patient was treated for four months with isoniazid and PAS and, unfortunately, no bronchogram was done. Subsequent sputum cultures for the past two years have been negative for tubercle bacilli, and the patient remains well.

A diagnosis of primary tuberculosis of the left upper lobe was made on the fourth patient *C. T.* in infancy. Two years later cultures were negative for tubercle bacilli, and fibrosis and calcifications were demonstrated roentgenographically in the left upper lobe. There was very little change on the roentgenograms during the following years, and the patient remained well. In 1956, twenty-six years after his known primary tuberculosis, he had pneumonia while working in a hospital. Because of his past history a gastric washing was cultured and a few colonies of tubercle bacilli were found. These organisms were susceptible to all drugs and were found to be virulent on guinea pig inoculation. After consideration, neither surgery nor antimicrobial therapy was carried out, and eighteen months later the patient is working, has had repeated sputum cultures negative for tubercle bacilli, and no change has been noted roentgenographically.

None of the cultures of these 4 patients showed many colonies of tubercle bacilli, but unfortunately no colony counts were done.

Drug susceptibility: In the entire group of 44 patients, only 2 instances of drug-resistant bacilli were found, in both cases resistance to streptomycin and, in each case, in cultures with a single colony of tubercle bacilli.

One infant, *R. C.*, with tuberculous meningitis and miliary tuberculosis treated with streptomycin and thiazolsulfone had cultures of tubercle bacilli from the cerebrospinal fluid and one initial positive culture of a gastric lavage which were completely susceptible to streptomycin. Because of persistent roentgenographic evidence of segmental obstruction, he was bronchoscoped twenty months after onset of his meningitis and was found to have a stenosed right upper lobe bronchus. Culture of the fluid obtained by bronchoscopy produced one colony of tubercle bacilli which was resistant to 100 γ of streptomycin. Sixteen days later another positive culture was obtained from bronchial fluid, again with one colony; unfortunately drug susceptibility was not recorded. Subsequent cultures of gastric and bronchial fluids were negative. The patient remains clinically well eight years later but with roentgenographic evidence of a contracted upper lobe.

A second infant, *A. P.*, with uncomplicated primary pulmonary tuberculosis, had an initial culture from gastric washings positive for tubercle bacilli completely susceptible to streptomycin which was followed by six subsequent negative cultures. A gastric lavage six months after admission and six days after a severe upper respiratory infection produced one colony of tubercle bacilli which was resistant to 100 γ of strep-

tomycin. Three subsequent cultures at monthly intervals were negative for tubercle bacilli. This patient received no specific antimicrobial therapy at any time and remains well seven years after the culture showing bacterial resistance.

Discussion

The fact that tubercle bacilli may be discharged a long time after the onset of primary tuberculosis without any roentgenographic or clinical changes in the patient seems to be established. Although no one has defined a "late positive," it seems to be generally accepted that an initial finding of tubercle bacilli or the reappearance of a positive culture more than six months after the diagnosis of primary pulmonary tuberculosis is the exception rather than the rule. It is difficult to determine the frequency with which "late positive" cultures occur, since children with primary tuberculosis are not usually followed by means of repeated gastric lavages or sputum examinations.

If the results of the study presented here are added to those of 3 other investigators (1, 4, 5), it may be seen that between 7.7 and 15 per cent of more than 700 children with primary tuberculosis excreted tubercle bacilli six months or more after the original diagnosis, almost always without any clinical or roentgenographic changes.

The significance of the delayed finding of tubercle bacilli in patients with clinically inactive primary tuberculosis should be considered both in relation to the importance of such findings in the epidemiology of tuberculosis and as to its possible influence on the prognosis of the individual patient. In addition it is important to discuss whether the finding of a few tubercle bacilli in itself always constitutes an indication for specific antimicrobial therapy.

It is impossible for anyone to do more than surmise to what extent children who occasionally excrete a few tubercle bacilli may be considered important in the spread of tuberculosis. Wallgren (8) believes that primary tuberculosis even in its early stages should not be considered contagious. Most investigators agree that not only do tuberculous children generally discharge fewer bacilli than older persons, but that excretion late in the course of primary tuberculosis usually yields only a few colonies on culture. In the series at Bellevue Hospital, unfortunately, colony counts were not regularly noted, but cultures positive for tubercle bacilli early in the course of primary tuberculosis usually showed more colonies than those which occurred late in children without symptoms. This was so generally accepted that a late culture containing 25 or more colonies of tubercle bacilli immediately raised the suspicion of a relapse or of the presence of active tuberculous disease of the upper respiratory tract. As the usual culture of tubercle bacilli appearing late in the course of primary tuberculosis without symptoms shows few colonies—often only one or two—it may be questioned whether such patients are a menace from an epidemiologic point of view. In any event, supervision and careful follow-up observation are essential.

Bacterial resistance was not an important consideration in the cultures positive for tubercle bacilli obtained late in the course of primary tuberculosis in Bellevue Hospital. The finding of one colony resistant to streptomycin in each of two cultures, one in a child who previously received antimicrobial therapy and the other in an untreated patient, is of doubtful significance.

The importance of the finding of even a few tubercle bacilli late in the course of primary tuberculosis must be considered in relation to the prognosis of the individual patient. Breton, Simonot, and Roger (9) express the opinion that the finding of tubercle bacilli means an active lesion susceptible of extension and is a menace for the future. Bertoye and his coworkers (7), Lowys and Nahum (10), and Dijkstra (11) agree with this interpretation and all advise a trial of antimicrobial therapy when tubercle bacilli are found. Bertoye and associates and Dijkstra agree that streptomycin may not cure the condition although bacilli may temporarily disappear from fluid obtained by gastric lavage. It is their opinion that a failure of medical therapy to stop all excretion of tubercle bacilli should lead to surgical consideration if two years have elapsed since the diagnosis of primary tuberculosis, provided the source of the bacilli can be localized by tomography, bronchoscopy, or bronchography.

Series of cases in which isoniazid has been used have not been reported. Only 2 of the Bellevue Hospital patients had been treated with isoniazid: one who had a culture of a gastric lavage positive for tubercle bacilli while on isoniazid and PAS therapy, which she had been receiving for nine months; and another who received isoniazid and thiazolsulfone for six months for endobronchial

tuberculosis and in whom tubercle bacilli were found on culture of gastric washings four months after therapy was discontinued, the organisms being fully susceptible to isoniazid. The question whether the late excretion of tubercle bacilli in primary tuberculosis will be seen less frequently in children treated by isoniazid can be settled only by a follow-up study of a large number of treated patients. The 44 patients in the Bellevue Hospital series have all been kept under supervision with an average follow-up period of more than nine years. In only one instance was the late finding of bacilli associated with an exacerbation of the tuberculous disease. The ability of tubercle bacilli to persist without evidence of clinical or roentgenographic changes and apparently without altering the prognosis of the disease is well shown in the 4 patients showing positive cultures of gastric fluid fourteen to twenty-five years after the first diagnosis of primary tuberculosis.

Alexander and her co-workers (12) recently reported 15 children with cultures of gastric washings positive for tubercle bacilli twelve months after a first diagnosis of tuberculosis. These patients were found as part of the Tuberculous Meningitis Prophylaxis Study of the United States Public Health Service and, on admission to the study, the cultures of the patients had all been negative. Ten of the children had received no specific therapy, but 5 had received approximately 5 mg. of isoniazid per kg. daily during the year of observation. Twelve of Alexander's 15 patients had normal roentgenographic findings throughout the year of observation. In contrast to this group, the majority of patients with late gastric washings positive for tubercle bacilli previously reported had roentgenographic evidence of primary pulmonary tuberculosis. The 44 patients from the Children's Chest Clinic at Bellevue Hospital had definite roentgenographic evidence of primary pulmonary tuberculosis with the exception of 2 children, one with a proved primary tuberculosis of the conjunctiva and one with tuberculosis of the tonsils and adenoids.

All investigators who have been interested in this subject believe that persistent excretion of tubercle bacilli is often associated with abnormalities of the bronchi. Studies in the pediatric age range have demonstrated the frequency of deformities of the bronchi as asymptomatic sequelae of primary tuberculosis (13–15). Studies in adults (16) have also suggested the importance of bronchography in revealing the source of tubercle bacilli in the presence of doubtful roentgenographic evidence. Schwartz (17) has emphasized repeatedly the importance of post-primary changes in the bronchi as foci of persistent disease. The possible role of intercurrent respiratory infection as a precipitating factor in the late excretion of tubercle bacilli is suggested in some cases. In addition, the series of cases from Bellevue Hospital here presented suggests that not only sequelae of endobronchial disease associated with primary tuberculosis should be sought, but also evidence of foci of tuberculosis draining into the upper respiratory tract.

The indications for treatment of a patient with healing primary tuberculosis who excretes a small number of tubercle bacilli unaccompanied by roentgenographic or clinical changes are not clear. It has been shown repeatedly that specific therapy has little effect on the nodal components of the primary complex or on the endobronchial lesions which they produce (15, 18), even if employed during the early stages of the disease. Their effective use in these areas years after the establishment of the primary tuberculosis is questionable. It is probable that the areas in which bacilli persist are areas in which drugs are inactive. Medlar (19) has stressed the fact that many patients with apparently inactive tuberculosis have closed necrotic foci. Russell and associates (20) have suggested that the intermittent excretion of small numbers of tubercle bacilli may be attributed in some cases to the occasional rupture of previously closed necrotic foci. Probably such an occurrence explains most of the single cultures positive for tubercle bacilli obtained in the 12 Bellevue patients two to twenty-five years after known primary tuberculosis. No evidence of tuberculosis was found in 3 of the 5 resections which were done in this group; the remaining 7 patients, only one of whom received specific therapy, have been followed for a further two to six years, with no change in clinical or roentgenographic status and no more cultures positive for tubercle bacilli. It would seem reasonable to treat or re-treat with antimicrobial therapy all children in whom tubercle bacilli reappear on culture within two years of a known primary focus in the hope of eradicating the lesion producing tubercle bacilli before it becomes a closed necrotic focus. Persons showing tubercle

bacilli on culture long after a known primary infection or with roentgenographic evidence suggesting arrested primary tuberculosis with or without previous endobronchial involvement should be subjected to careful study by bronchoscopy, bronchography when necessary, and repeated bacteriologic examinations. In the occasional cases in which roentgenograms are normal, otolaryngologic examination should be undertaken. In any event, such cases should be carefully studied prior to a decision regarding chemotherapy or surgery.

Summary

The significance of the excretion of tubercle bacilli in children six months old or more after the onset of primary tuberculosis has been investigated by a review of the literature and of the experience in the Chest Clinic of the Children's Medical Service of Bellevue Hospital between March, 1947, and July 1, 1956.

Tubercle bacilli were cultured from gastric or bronchial fluids six months to more than twenty-five years after the initial diagnosis of primary tuberculosis in 44 patients. No significant roentgenographic changes accompanied the late exretion of tubercle bacilli. Twenty-seven patients had endobronchial disease proved by endoscopy, and an additional 8 had roentgenographic or bronchographic findings suggestive of previous endobronchial involvement. Foci of tuberculosis draining into the upper respiratory tract, usually in tonsils or adenoids, were found in 11 of the 44 patients.

Sumario

Excreción Tardía de Bacilos Tuberculosos en la Tuberculosis Primaria

Se ha investigado la importancia que atañe a la excreción de bacilos tuberculosos en los niños de seis meses o más de edad después de la iniciación de la tuberculosis primaria por medio de un repaso de la literatura y de lo observado en la Clínica Torácica del Servicio Médico de Niños del Hospital Bellevue entre marzo de 1947 y el 1° de julio de 1956.

En 44 enfermos se cultivaron bacilos tuberculosos de los líquidos gástricos o bronquiales de seis meses a más de veinticinco años después del diagnóstico inicial de tuberculosis primaria. La excreción tardía de bacilos tuberculosos no fué acompañada de mayores alteraciones radiográficas. Veintisiete enfermos tenían enfermedad endobronquial comprobada con la endoscopia y otros 8 mostraron signos roentgenográficos o broncográficos indicativos de previa invasión endobronquial. En 11 de los 44 enfermos se descubrieron focos tuberculosos que drenaban en las vías aéreas superiores, por lo general las amígdalas o las vegetaciones adenoideas.

Resume

Excrétion tardive de bacilles tuberculeux dans la tuberculose primaire

La signification de l'exrétion de bacilles tuberculeux chez des enfants de six mois ou plus après le début d'une primo-infection tuberculeuse a été étudiée d'après les rapports publiés et l'expérience acquise dans la clinique des Maladies du Poumon du Service de Pédiatrie médicale de l'Hôpital Bellevue (New York) de mars 1947 au 1er juillet 1956.

Les bacilles tuberculeux ont été cultivés à partir d'expectoration bronchique ou de liquide gastrique chez 44 patients de six mois à plus de vingt-cinq ans après le diagnostic initial de primo-infection tuberculeuse. Aucune modification radiologique significative n'était associée à cette exrétion tardive de bacilles tuberculeux. Vingt-sept malades présentaient une localisation endobronchique de l'affection, décelée par endoscopie, et 8 autres présentaient des signes radiologiques ou bronchographiques indiquant une atteinte endobronchique antérieure. Des foyers de tuberculose, avec drainage par les voies respiratoires supérieures, et qui affectaient les amygdales ou les végétations adénoïdes, ont été découverts chez 11 de ces 44 malades.

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THE IMPORTANCE OF PRERESECTION DRUG THERAPY FOR PULMONARY TUBERCULOSIS[1]

DAVID V. PECORA

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Introduction

Although antituberculous drugs have been employed for a considerable period there is still disagreement regarding their precise value prior to pulmonary resection. Even among those who accept the value of such therapy, opinions vary regarding its optimal duration. A limiting factor is the belief that prolonged drug therapy may foster the emergence of resistant bacterial strains. As the length of recommended preresection drug therapy varies from a few days to a number of months, it was decided to undertake a study in an attempt to formulate a rational basis for determining the optimal period for such therapy.

Materials and Methods

The pulmonary resections for tuberculosis performed at the Ray Brook State Tuberculosis Hospital between January 1, 1950, and January 1, 1957, were reviewed with the object of determining the effect of preoperative antituberculous drug therapy upon the bacteriology of the sputum and resected tissue. An attempt was made to evaluate the importance of other factors, such as extent of disease and presence or absence of cavity. Sputum bacteriology was compared to that of the resected tissue. Finally, an attempt was made to determine the frequency of emergence of detectable numbers of resistant organisms during the course of uninterrupted preoperative multiple-drug therapy.

In a minority of instances at the start of therapy, patients harbored organisms resistant *in vitro* to one or more of the drugs administered. In these instances the drugs to which the organisms proved resistant are not listed in the various graphs because of the writer's belief that in these circumstances the drugs are ineffective therapeutically (1).

Tubercle bacilli were considered susceptible to streptomycin, para-aminosalicylic acid (PAS), or isoniazid if they grew in the control medium and failed to grow in the presence of concentrations of 10 γ of streptomycin or PAS per ml. and 5 γ of isoniazid per ml. Susceptibility studies were performed according to the technique described previously (1).

The presence or absence of cavitary disease was determined from the resected tissue by the examining pathologist. So-called "filled-in cavities" containing caseous material were not classified as cavitary unless they also contained an air space as evidence of a free communication with a bronchus.

Results

The upper part of figure 1 demonstrates the effect of various drug regimens upon the yield of culturable tubercle bacilli from the sputum in 328 resections. It should be noted that the great majority of patients had culturable tubercle bacilli in the sputum at the commencement of these courses of drug therapy. Some of them had received previous drug therapy. Patients in whom resistant organisms emerged while they received these courses of therapy were eliminated from the graphs in order to eliminate the influence of the emergence of resistant strains upon the results. These patients were considered later. Furthermore, no attempt was made to distinguish between 1 gm. of streptomycin given two or three times weekly, or daily streptomycin. Para-aminosalicylic acid and isoniazid were administered daily, usually in doses of 12 gm. and approximately 300 mg., respectively.

Two patients receiving streptomycin as the only effective drug failed to develop noninfectious sputum after one and five months, respectively. These patients are not included in figure 1. Para-aminosalicylic acid did not prove as successful as isoniazid, and isoniazid was probably less effective than multiple-drug therapy in eliminating culturable organisms from the sputum. Indeed, the results for the single "effective" drugs[2] are even less favorable than the upper part of figure 1 would seem to indicate since the sputum of a considerable proportion of the patients in those categories was rendered free of culturable tubercle bacilli by surgery. Surgery performed upon patients with culturable tubercle bacilli in the sputum is indicated at the bottom of figure 1. Relatively few patients receiving multiple-drug

[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

[2] *See* "Materials and Methods."

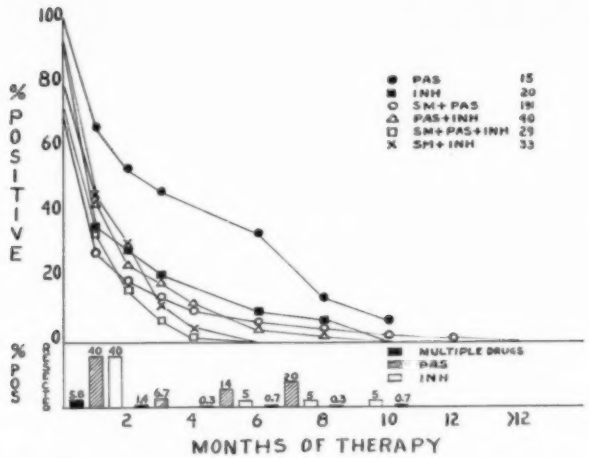

FIG. 1. The upper graph indicates the effect of antituberculous drugs upon the sputum culture for tubercle bacilli. The abscissa indicates months of continuous therapy; the ordinate, per cent of positive cultures. The lower graph indicates the percentage of the resections which were performed during each two-month period upon patients with infectious sputum and which were responsible for converting the sputum to a noninfectious state during single and multiple drug therapy.

The only drugs listed are the drugs to which the patients' tubercle bacilli were demonstrably susceptible *in vitro*. If the patients' tubercle bacilli were resistant to a drug, it is omitted from consideration and not listed. The number of patients in each category is listed after each drug regimen.

SM—streptomycin; PAS—para-aminosalicylic acid; INH—isoniazid.

therapy were operated upon while the sputum contained culturable tubercle bacilli.

This difference in the "conversion rate" of the sputum with single and multiple-drug regimens cannot be explained on the basis of the emergence of resistant organisms, since only patients whose organisms remained susceptible to the indicated drugs during the whole course of therapy were included in figure 1. Therefore, it appears reasonable to conclude that these combinations of drugs had a greater therapeutic effect than a single drug.

The superiority of isoniazid-containing regimens over streptomycin and PAS conforms with the results reported by Livings (2). In figure 1, none of the patients who received streptomycin–isoniazid or the triple-drug regimen had culturable tubercle bacilli in the sputum after six months of therapy.

The percentage of specimens yielding tubercle bacilli microscopically and by culture at various periods is presented in figure 2. The higher yield of bacilli microscopically has been reported by Hughes (3). There does not appear to be a great difference in the yield of bacilli by this method from patients receiving single- as compared to multiple-drug therapy; nor does the length of therapy have a great effect upon the percentage of specimens containing microscopically detectable bacilli. On the other hand, the yield of culturable bacilli is affected more by multiple-drug than by single-drug therapy, and is generally further favorably influenced by increased duration of therapy. An apparent exception to the latter statement is the PAS group. Two previously mentioned patients treated by streptomycin yielded culturable tubercle bacilli after one and five months, respectively. When multiple-drug therapy is employed, the maximal effect appears to have occurred by approximately the eighth month.

When figure 1 and figure 2 are compared, it is evident that the recoverability of culturable organisms is higher from the resected tissue than from the sputum, an observation which has been made by many others. However, the difference in yield of culturable organisms from these sources generally appears to be about 10 per cent.

Attempts to correlate the bacteriology of the

sputum or resected tissue with the amount of pulmonary disease at the commencement of therapy were unsuccessful in this group of patients. However, this correlation was established in a larger group of patients by Livings (2), who showed that conversion of sputum from an infectious to a non-infectious state was definitely influenced by the extent of pulmonary disease.

The bacteriologic response of cavitary and non-cavitary disease is summarized in figure 3, which indicates the percentage of resected lesions yielding culturable tubercle bacilli after various per-

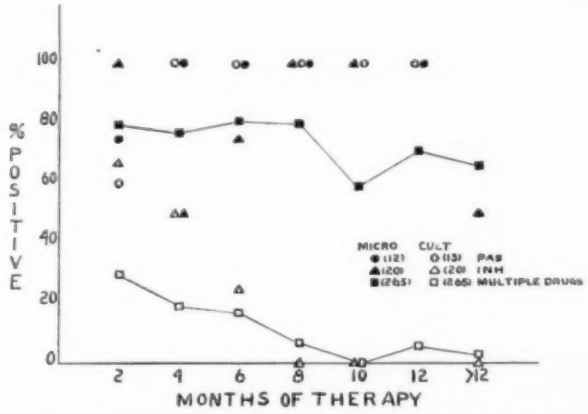

FIG. 2. The effect of antituberculous drugs upon the percentage of resected specimens yielding tubercle bacilli microscopically and by culture after various periods of drug therapy. The only drugs listed are the drugs to which the patients' tubercle bacilli were demonstrably susceptible *in vitro*. If the patients' tubercle bacilli were resistant to a drug, it is omitted from consideration and not listed.

The number of specimens in each category is listed after each drug regimen. Results of multiple-drug therapy are connected for easy reference.

MICRO—microscopically; CULT—by culture

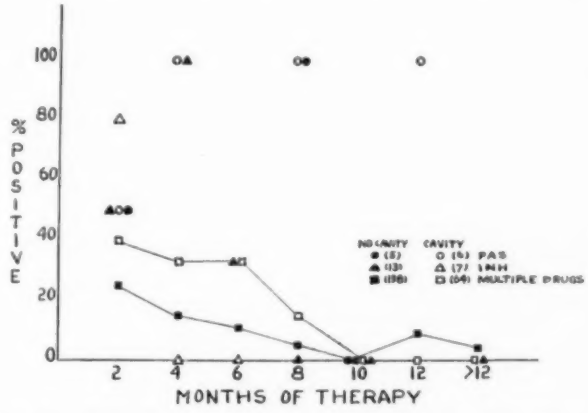

FIG. 3. The effect of cavitary disease upon the percentage of resected specimens yielding tubercle bacilli by culture after various periods of drug therapy. The number of specimens in each category is listed after each drug regimen. The only drugs listed are the drugs to which the patients' tubercle bacilli were demonstrably susceptible *in vitro*. If the patients' tubercle bacilli were resistant to a drug, it is omitted from consideration and not listed.

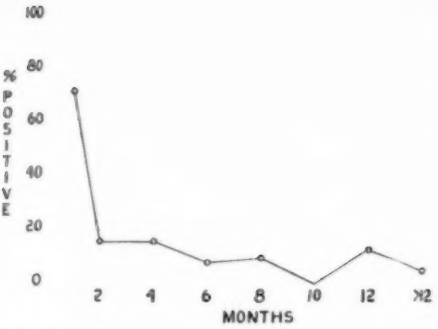

FIG. 4. Correlation of the duration of noninfectious sputum with the percentage of resected specimens yielding culturable tubercle bacilli. The abscissa indicates the number of months that the sputum has been free of culturable tubercle bacilli; the ordinate, the percentage of specimens of resected pulmonary tissue yielding culturable tubercle bacilli.

iods of antituberculous drug therapy. Two patients who received streptomycin, one with and one without cavity, had infectious specimens after one and five months, respectively. Generally, noncavitary disease is influenced more favorably. However, the difference in response of cavitary and noncavitary disease to continued multiple-drug therapy decreases until at ten months the results are quite similar. Indeed, beyond eight months, and for a period well beyond twelve months, none of 25 cavitary lesions examined yielded culturable tubercle bacilli. This similarity after ten months of therapy cannot be attributed to the elimination of patients with infectious sputum by surgery, since, as indicated in figure 1, few patients receiving multiple-drug therapy were operated upon in the infectious state.

The relationship between the duration of noninfectious sputum and the bacteriology of the resected specimen is indicated in figure 4, which includes data from 379 resections. As indicated by Shefrin (4), approximately 10 per cent of the resected specimens yielded culturable tubercle bacilli after the sputum had been noninfectious for four months.

To determine the incidence of emergence of bacterial resistance during uninterrupted therapy with various combinations of streptomycin, PAS, and isoniazid, the bacteriology of 224 resections upon patients with drug-susceptible tubercle bacilli in the sputum at the start of therapy was reviewed. During the course of therapy, resistant organisms were detected in the sputum and/or resected tissue of one (1.2 per cent) of 82 patients who received various combinations which included isoniazid, and 7 (5 per cent) of 142 patients who received streptomycin and PAS. It is interesting to note that resistance to two drugs (streptomycin and PAS) had occurred in only 2 patients when first detected. Resistance to isoniazid occurred on one occasion; to streptomycin, in 7 instances; and to PAS, in 2 instances. The influence of morphology is indicated by the fact that 5 (7.9 per cent) of 63 patients with cavitary disease compared with 3 (1.9 per cent) of 161 patients with noncavitary disease developed resistant organisms.

DISCUSSION

The data presented assumes significance if it is realized that since January 1, 1950, the writer has not encountered any tuberculous complications within one month following pulmonary resection—and only 2 per cent of late tuberculous complications—in the group of patients from whose sputum and resected tissue tubercle bacilli were not cultured at the time of surgery. Moreover, the complications which did occur (bacterial or roentgenographic relapse) were transient. Admittedly, the methods employed in culturing resected tissue are subject to sampling errors, but they seem adequate enough to provide a good correlation with surgical results. This relationship having been established, it should be possible to predict accurately from the data presented the results of preoperative drug therapy and the optimal time for resection.

First of all, it appears that the likelihood of the appearance of detectable numbers of drug-resistant tubercle bacilli during continuous multiple-drug therapy has been overstressed, especially when regimens include isoniazid. There is every reason to believe that the results in previously untreated tuberculosis might be even more favorable than in this series which included re-treatment cases. Even in those instances in which resistant organisms were recovered, the organisms were resistant to only one drug in the majority of the cases. It has been shown that one effective drug provides adequate protection against the occurrence of early postresection tuberculous complications (1), certainly long enough to per-

mit susceptibility studies upon the bacilli recovered from the resected tissue and to permit any indicated changes in the postoperative drug regimen. Moreover, there were no tuberculous complications in the 2 patients who harbored organisms resistant to the two (and only) drugs which were administered at the time of resection. That these resections were without complication is probably attributable to the fact that the bacterial population in patients with noninfectious sputum usually has been so reduced that the risk with good surgical technique is slight even without the protection afforded by antituberculous drugs.

It would appear advisable to perform pulmonary resection after about four months of multiple-drug therapy upon any patients, otherwise suitable, whose sputum still contains culturable tubercle bacilli, since the probability of subsequent conversion to a noninfectious state is slight. Although in such cases thought might be given to the substitution of two new drugs prior to resection if there is a considerable amount of disease, this is probably not necessary if the organisms recovered from the sputum at the commencement of drug therapy (or more recently) were susceptible to the prescribed regimen.

If the patient's sputum becomes noninfectious within four months after the start of combined drug therapy, resection should be delayed until the sputum has been noninfectious for at least four months unless there is a bacterial or roentgenographic relapse, which is rare under these circumstances. Such relapses may herald the appearance of resistant organisms. If cavitary disease is present, in the absence of relapse, resection should be delayed until the sputum has been noninfectious for approximately ten months. However, preresection therapy should be continued as long as there is appreciable roentgenographic improvement.

Although at present few would routinely prescribe single-drug therapy, many inadvertently do so if they blindly add one new drug to existing regimens when the emergence of resistant bacilli is suspected. Single-drug therapy yields inferior results not only because of an early tendency to develop resistant variants but also because the therapeutic effect appears to be less.

Accumulated evidence would seem to indicate that the extent of pulmonary disease at the inception of drug therapy bears a direct relationship to the bacteriologic and roentgenographic results. It would therefore appear logical to treat patients with advanced disease longer than those with minimal disease. This offers the added advantage of often reducing the amount of surgery which might be required.

The superiority of isoniazid in combination with streptomycin and/or PAS over streptomycin and PAS alone seems well established. In this study, culturable tubercle bacilli were not obtainable from the resected tissue of any of 53 patients who received combinations of drugs including isoniazid for more than seven months.

Although it seems advantageous to prescribe prolonged therapy with isoniazid in combination with streptomycin and/or PAS prior to pulmonary resection, it is recognized that decisions cannot be based entirely upon bacteriologic consideration and may require modification because of social and economic factors.

Summary

Seven years' experience with pulmonary resection for tuberculosis is reviewed in an attempt to determine the optimal preoperative therapy with various combinations of streptomycin, para-aminosalicylic acid, and isoniazid. The best results were obtained with combinations which included isoniazid. Sputum bacteriology is an important guide to the bacteriology of the tissues. The dangers of prolonged preoperative antituberculous therapy have been exaggerated. On the other hand, combined antituberculous drug therapy of four to ten months or more tends to ensure a lower incidence of tuberculous complications than short-term therapy.

Sumario

La Importancia de la Farmacoterapia Pre-Resección en la Tuberculosis Pulmonar

Repásanse siete anos de experiencia con la resección pulmonar por tuberculosis tratando de determinar la terapéutica preoperatoria óptima con varias combinaciones de estreptomicina, ácido para-aminosalicílico e isoniacida. Se obtuvieron los mejores resultados con las combinaciones que comprendían la isoniacida. La bacteriología del esputo es una guía importante para la de los tejidos. Se han exagerado los peligros que entraña la prolongada terapéutica antituberculosa preoperatoria. En cambio, la farmacoterapia antituberculosa combinada de cuatro a diez meses o más de duración tiende a asegurar una incidencia más

baja de complicaciones tuberculosas que la terapéutica a brave plazo.

Resume

L'importance du traitement médicamenteux prérésectionnel dans la tuberculose pulmonaire

L'auteur passe en revue sept années d'expérimentation de la résection pulmonaire dans la tuberculose dans le but de déterminer le traitement préopératoire le plus favorable en utilisant diverses combinaisons de streptomycine, d'acide para-aminosalicylique et d'isoniazide. Les meilleurs résultats ont été obtenus avec le traitement combiné comprenant l'isoniazide. La bactériologie du crachat est un guide important pour la bactérlologie tissulaire. Les dangers de la thérapeutique antituberculeuse préopératoire prolongée ont été exagérés, d'autre part, les traitements combinés antituberculeux prolongés pendant quatre à dix mois ou plus tendent à assurer une incidence plus réduite des complications tuberculeuses que le traitement à court terme.

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INFECTIVITY AND IMMUNOGENICITY OF MYCOBACTERIUM X IN MICE[1, 2]

EMANUEL SUTER[3] AND ANNE K. STRAIN

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INTRODUCTION

A pleomorphic organism, tentatively called *Mycobacterium X*, isolated from mouse brain was characterized by Vischer and associates (1). The pathologic changes induced in mice by this organism were described by Pappenheimer and co-workers as being minute, proliferative granulomas (2). It is noteworthy that no caseous necrosis was observed in these lesions. Acid-fast organisms could readily be demonstrated within cells, but they were usually single and never formed compact colonies such as are found in mouse tissues infected with a virulent avian strain. It was suggested that *Mycobacterium X* represents an attenuated type of avian tubercle bacilli, although this conclusion is not convincing. It should be added that guinea pigs, rabbits, and chickens appeared to be completely resistant to this organism.

In tissue culture experiments, it was noted that attenuated and virulent tubercle bacilli were inhibited within monocytes derived from guinea pigs which had been infected with *Mycobacterium X* (3). These findings suggested that *Mycobacterium X* has immunogenic properties. Since there is no conclusive histologic evidence for multiplication of this organism in mouse tissues, a study was undertaken to determine the relationship between its immunogenic capacity and its behavior in the tissues. It was expected that information about this relationship would help to clarify the problem whether or not multiplication of attenuated organisms in the host's tissues is essential for the establishment of immunity to infection.

MATERIALS AND METHODS

Animals: Swiss white mice of the Schwentker strain were used. The animals weighed from 15 to 18 gm. They were fed pellets and water ad libitum and were housed in metal cages or in glass jars.

Cultures and their maintenance: The following cultures were used in this study: *Mycobacterium X* isolated by Vischer and associates (1), BCG,[4] H37Rv and Vallée.[5] Cultures were maintained by weekly transfers in Dubos' liquid medium containing Tween® 80 and bovine albumin. Unless stated otherwise, the cultures were between seven and ten days old when used for vaccination or infection.

Vaccination and infection: All injections of 0.1 ml. were done by the intravenous route.

Enumeration of bacilli: The turbidity of the cultures was read at $\lambda = 620$ mμ. Total counts were done in the Petroff-Hauser counting chamber. For viable counts 0.1 ml. samples of tenfold serial dilutions were spread over the surface of small Petri dishes containing solid medium with oleic-acid–albumin and 0.1 per cent glucose. For the determination of viable bacilli in the tissues, the mice were killed with chloroform, and livers and spleens were removed aseptically. Part of each organ was weighed and then ground in a mortar with alundum. To the ground tissue, 1.0 ml. of diluent was added per 100 mg. of tissue. Aliquots of 0.1 ml. were spread on the surface of solid media (4). The number of viable units per 100 mg. of tissue was then obtained.

Challenge infection and evaluation of degree of immunity to infection: Control mice, and mice vaccinated with BCG or *Mycobacterium X* were infected with either H37Rv or with the Vallée strain. In experiments in which the mice were infected with Vallée, mortality served as the indicator of the severity of the infection. Mice infected with H37Rv were killed approximately five weeks after infection, and the bacillary population in tissues, especially the lungs, was established in histologic sections by counting acid-fast organisms within lesions. One hundred cells containing bacilli were counted and grouped according to the number of intracellular bacilli. An estimation was also obtained of the number of microscopic fields which had to be searched in order to find 100 cells containing bacilli. According to findings by Siebenmann (5), the number of stainable tubercle bacilli visible in tissues can be used as an

[1] From the Department of Microbiology, College of Medicine, University of Florida, Gainesville, Florida.

[2] This work was supported by Grant E-1302 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[3] Markle Scholar in Medical Science.

[4] BCG was obtained from The Henry Phipps Institute, Seventh and Lombard Streets, Philadelphia, Pennsylvania.

[5] H37Rv and Vallée strains were obtained from Dr. R. J. Dubos's laboratory at The Rockefeller Institute of Medical Research, 66th Street and York Avenue, New York 21, New York.

indicator of the severity of an infection and is altered by vaccination or chemotherapy.

Results

Fate of Mycobacterium X in tissues of normal mice: Groups of 10 mice each were injected intravenously with 6.5×10^6 and 6.5×10^4 organisms, respectively, of a nine-day-old culture. At various intervals, animals of both groups were killed with chloroform and the numbers of viable organisms were determined in the livers and the spleens. As may be seen from table 1, the bacterial populations remained stable for seven days and then began to decline slowly. The total number of organisms found in livers and spleens after twenty-four hours was 39 per cent of the inoculum in mice injected with the large dose and 59 per cent in those injected with the smaller one. After seven days the figures were 26 and 32 per cent, respectively. The fact that in both groups a similar percentage of the injected organisms was recovered from the two tissues indicates that no or only little multiplication occurred in the tissues.

It has been observed earlier (1) that *Mycobacterium X* would grow in the Tween-albumin medium at first as long bacilli and then disintegrate into coccobacillary or granular forms. To compare the fate of these two forms in the tissues, cultures of two and eight days of age were injected into mice, the first culture consisting almost exclusively of long and slender bacilli and the second, of granular forms. The two cultures were adjusted to contain similar total bacillary counts, and 0.1 ml. was injected intravenously; the two suspensions contained 3×10^6 and 2×10^6 organisms, respectively. The data in table 2 show that the bacillary forms disappeared somewhat more rapidly than the granular ones. There was no significant evidence that there was a real difference between the two cultures.

TABLE 1

Number of Viable Organisms Recovered from Livers and Spleens of Mice at Various Intervals of Time After Intravenous Infection with Mycobacterium X

Time After Infection	Number of Organisms per 100 Mg. of Tissue × 100			
	Group A*		Group B	
	Liver	Spleen	Liver	Spleen
days				
1	1,300	690	14	40
	1,400	220	16	—
7	750	570	12	7.2
	1,100	490	10	7.0
29	46	34	1.8	1.3
	84	23	3.1	2.2
46	30	49	1.8	0.9
	12	20	1.3	1.5
89	24	11		
	12	7		

* Mice of group A were infected with 6.5×10^6 organisms and those of group B with 6.5×10^4 organisms.

TABLE 2

Number of Viable Organisms Recovered from Livers of Mice at Various Intervals of Time After Intravenous Infection with Bacillary and Granular Forms of Mycobacterium X

Time After Infection	Number of Organisms per 100 Mg. of Tissue × 100	
	Group A*	Group B
days		
1	1660/2100/2200	1700/2000/2200
8	500/ 600/ 700	1800/1900/2200
15	170/ 320/ 660	470/ 580/ 900

* Group A was injected with 3×10^6 organisms of a 2-day-old culture (bacilli) and the mice of group B with 2×10^6 organisms of an 8-day-old culture (granular forms).

The comparison of Mycobacterium X and BCG as protective antigens: Three different methods were employed to evaluate the effect of infection with *Mycobacterium X* upon the course of a subsequent challenge infection with virulent tubercle bacilli:

A. The effect of vaccination upon the bacterial population within the pulmonary lesions after challenge: In this experiment, the mice were challenged with a number of H37Rv bacilli which caused a slowly progressing but not a fatal infection in mice. The mice were killed five weeks after infection, and sections were prepared from the lungs. The bacterial population was established by counts, and the histologic findings were evaluated on an arbitrary scale from 1 to 10. The data obtained in a typical experiment are set forth in table 3. According to these results, vaccination with BCG as well as with *Mycobacterium X* restricted the bacterial population of H37Rv in the lesions to a significantly lower level than in tissues of control animals. The number of microscopic fields, which have to be counted to find 100 infected cells in the section, gives information on the distribution of bacilli throughout the tissues

TABLE 3

NUMBER OF INTRACELLULAR TUBERCLE BACILLI IN PULMONARY LESIONS OF NORMAL MICE AND MICE VACCINATED WITH MYCOBACTERIUM X OR BCG AFTER INTRAVENOUS INFECTION WITH H37Rv.*

Group	Per Cent of Phagocytes Containing the Following Number of Bacilli				Number of Fields Counted for 100 Infected Phagocytes	Histologic Rating
	1–2	3–5	6–10	>10		
Controls	65.4 ± 2.63	28.0 ± 2.63	6.1 ± 1.13	0.5 ± 0.16	336 ± 47.96	100
Mycobacterium X	82.2 ± 1.19	15.7 ± 1.53	2.0 ± 0.83	0.1 ± 0.96	1,084 ± 297.1	40
BCG	82.8 ± 2.0	14.7 ± 1.27	1.8 ± 0.84	0.7 ± 0.6	3,473 ± 753.2	32

* The animals were challenged 41 days after vaccination and killed 35 days later.

and serves as an indication of the severity of the infection. As expected from bacterial counts, lesions were less frequent and smaller in the vaccinated animals than in the controls.

B. The effect of vaccination on the course of slowly progressing infection: By challenging the mice with a large dose of H37Rv the infection of the controls was fatal. The results summarized in table 4 show the effect of vaccination on such an infection. Again, *Mycobacterium X* appears to be as effective in increasing the resistance of mice to a virulent infection as is BGG.

C. The effect of vaccination on the course of a rapidly progressing infection: About 10^7 organisms of the Vallée strain injected intravenously cause a rapidly progressing infection, of which all control mice die within twenty to twenty-five days. The effect of vaccination with BGG or *Mycobacterium X* is shown in table 5. The data show that *Mycobacterium X* was an effective protective antigen and that dilution diminished its effectiveness to a greater extent than was the case with BGG. The data also show that heat-killed organisms were less effective than living ones.

TABLE 4

MORTALITY OF MICE INFECTED WITH H37Rv AND INFLUENCE OF VACCINATION WITH MYCOBACTERIUM X OR BCG*

Days After Challenge	Numbers of Survivors				
	Controls	*Mycobacterium X*			BCG
		Undiluted	1:10	1:100	
30	13/15	14/14	14/14	16/16	14/14
40	11	14	13	15	14
50	9	14	13	15	14
60	6	14	11	15	13
70	3	14	10	13	12
80	2	11	8	7	10
90	0	11	7	7	9

* The mice were vaccinated by the intravenous route with 0.1 ml. of undiluted BCG (containing 7.5×10^6 organisms) and undiluted and diluted culture of *Mycobacterium X* (containing 1.4×10^8 organisms in the undiluted inoculum), respectively. The animals were challenged 4 weeks after vaccination with 0.2 ml. of a culture of H37Rv.

DISCUSSION

The results presented in this paper are evidence that *Mycobacterium X* is an effective protective antigen in mice and induced in them a state of increased resistance to a challenge infection with human or bovine tubercle bacilli. The degree of protection achieved was close to that usually obtained after vaccination with BCG. This seemed to be the case irrespective of whether mortality as a result of a virulent progressive reinfection or the extent of the lesions induced by a somewhat attenuated type of reinfection served as a measure of resistance of the host.

The findings also demonstrated that *Mycobacterium X* did not multiply to any measurable extent in the tissues of the mouse. This is inferred from the fact that the number of organisms in the tissues remained relatively constant for about ten days and declined only slowly thereafter. Three months after infection approximately one-hundredth of the original population was still present. Furthermore, if a smaller inoculum was used, the bacterial population in the tissues reached a level which was proportionally lower. In contrast, if a strain of attenuated tubercle bacilli is injected into mice, the increase of the bacterial population in the tissues continues over a prolonged period of time, if a small inoculum is used (4). Compared with the completely avirulent strain H37Ra, *Mycobacterium X* appeared to persist longer in the tissues. However, it is difficult to exclude any multiplication of *Mycobacterium X* provided it is compensated by a nearly equal death rate in the tissues.

TABLE 5

MORTALITY OF MICE INFECTED WITH VALLÉE AND INFLUENCE OF VACCINATION WITH VARIOUS DOSES OF BCG AND MYCOBACTERIUM X*

Group	Experiment A†	Experiment B			
	Number of Survivors After Days				
	25	10	15	20	30
Control	0/10	9/10	8/10	0/10	0/10
BCG, undiluted	18/18	10/10	10/10	10/10	7/10
BCG, 1:100	14/19	—	—	—	—
Mycobacterium X undiluted	15/19	10/10	10/10	10/10	3/10
Mycogacterium X, 1:100	2/20	—	—	—	—
Mycobacterium X undiluted heat-killed	—	10/10	10/10	5/10	0/10

* The mice were challenged 3 weeks after the immunizing injection.

† Experiment A was done in the laboratory of Dr. H. Bloch, Department of Microbiology, University of Pittsburgh Medical School, Pittsburgh 13, Pennsylvania.

It is suggested that a more detailed study of the fate of *Mycobacterium X* in the tissues of the host by means of viable counts and enumeration of stainable bacilli in tissue sections may throw some light on the controversial question whether bacterial multiplication is essential for the induction of immunity in tuberculosis (6). The present experiments are too limited to allow any firm statement in this respect. The advantage of using *Mycobacterium X* for such a study lies in the fact that its multiplication is limited by some inherent factor of the host's tissues rather than by an extraneously introduced inhibitor.

The results of the immunization experiments indicated that *Mycobacterium X* must share some common antigen with human or bovine strains of Mycobacteria. This was also supported by experiments in which guinea pigs infected with *Mycobacterium X* were tested for skin hypersensitivity to tuberculoproteins derived from human tubercle bacilli.[6] These guinea pigs showed a typical delayed response to tuberculoprotein five weeks after infection, but the reactions were less intense than in guinea pigs vaccinated with BCG at the same time. There was little doubt that the reaction was of the delayed type.

Finally, the similarity of behavior in tissues of granular and bacillary forms of *Mycobacterium X* is worthy of attention. Progressive shortening of Mycobacteria under adverse environmental conditions has been called regression and has been assumed to result in qualitative changes of the organisms (7). From the present experiments, it appears that short as well as long forms had similar chances of surviving in the tissues, and no evidence was found to support the view that either of the two forms possessed greater vitality or resistance. It would seem that fragmentation of the bacilli yields units of full biologic potential (8).

[6] These experiments were done by Dr. Stephen V. Boyden, Tuberculosis Immunization Research Center, % State Serum Institute, Copenhagen, Denmark.

SUMMARY

The fate of *Mycobacterium X* in the liver and spleen of the mouse has been compared with its effectiveness as a protective antigen. This organism did not appear to multiply in the tissues of the mouse, but it induced infection immunity which was equal, or only slightly inferior, to the state of resistance induced by vaccination with BCG. Long bacillary and short coccoidal forms of *Mycobacterium X* behaved similarly in the tissues. Their comparative immunogenicity has not been determined.

SUMARIO

Infectividad e Inmunigenicidad del Mycobacterium X en los Ratones

Se ha comparado lo sucedido al *Mycobacterium X* en el hígado y bazo del ratón con su eficacia como antígeno protector. Este microbio no parece multiplicarse en los tejidos del ratón, pero indujo inmunidad por infección que fué igual, o apenas ligeramente inferior, al estado de resistencia inducido por la vacunación con BCG. Las formas bacilares largas y las cocoideas cortas del *Mycobacterium X* se comportaron de modo semejante en

los tejidos. No se ha determinado su inmunigenicidad comparativa.

Resume

Contagiosité et immunogénicité du Mycobactérium X chez la souris

Une étude comparative du sort du *Mycobactérium X* dans le foie et la rate de la souris et de son efficacité comme antigène ,protecteur a été effectuée. Cet organisme ne paraissait pas se reproduire dans les tissus de la souris, mais il provoquait une immunité contre l'infection égale, ou légèrement inférieure, à l'état de résistance déterminé par la vaccination par le B.C.G. Les types à bâtonnet long ou coccicoïde court du *Mycobacterium X* se comportaient de la même manière dans les tissus. Leur immunogénicité comparative n'a pas été déterminée.

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THE LYMPHATICS WHICH DRAIN THE POTENTIAL SPACE BETWEEN THE VISCERAL AND THE PARIETAL PLEURA[1, 2, 3]

HUGH E. BURKE

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Introduction

Few investigators seem to recognize that lymphatics play an indispensable part in the removal of particles and proteins from spaces lined with serous membranes, as Courtice and Simmonds (1) recently pointed out. Lack of appreciation of this fact and failure to make use of certain experimental findings with respect to the lymph channels, by which particulate matter is removed from the pleural spaces, appear to be responsible for some of the difficulties encountered in attempts to find solutions for unsolved problems in the pathogenesis of disease in man.

This study, which stems from certain findings that were described by the present investigator (2, 3) in 1940 and one observation that was reported in 1954, is concerned with: the lymph nodes to which the lymphatics of the potential space between the visceral and the parietal pleura drain and some of the factors which effect lymph transport from the pleural space.

Review of the Literature

In 1894, Starling and Tubby (4) pointed out that it had been known for a long time that solid particles are absorbed from serous cavities. From experiments carried out on anesthetized dogs, they concluded: that coloring matters in solution placed in serous cavities are absorbed directly and rapidly by the blood vessels, and that absorption from, and secretion into, the pleural cavity is not simply a matter of osmosis.

Karsner and Swanbeck (5) reported, in 1920, that suspensions of carmine and of lampblack introduced into the pleural cavities of cats are taken up by the endothelial cells of the pleura and are transported in these or other phagocytic cells to lymph nodes in the mediastinum and at the hilus of the lung and, on occasion, to a lymph node in the abdomen lying over the aorta and just beneath the diaphragm.

In a review of the physiology of the serous membranes which was published in 1926, Cunningham (6) used findings with respect to the peritoneal cavity as a basis for his observations. He drew attention to the fact that, as a result of the work of early investigators, notably Von Recklinghausen, Bichat, and Henle, two main concepts concerning the peritoneal cavity emerged: one, that it is an integral part of the lymphatic system; the other, that it is lined by a characteristic type of flattened cells. After pointing out that experimental findings show that particles introduced into the peritoneal space are removed by way of lymph channels, are transported to the anterior mediastinal nodes, and may subsequently be distributed throughout the body, he noted that some evidence indicates that respiratory movements play a part in the transfer of particulate matter from the peritoneal cavity to the diaphragmatic lymphatics. When describing the absorption of fluids from serous cavities, he stated that, although early workers considered the serous cavities to be part of the lymphatic system, some investigators have failed to find evidence to support this view, and certain recent findings seem to indicate clearly that blood vessels play an important part in the removal of fluids from the peritoneal cavity and also from the pleural cavities.

Several papers relating to absorption from the pleural cavities and from the peritoneal cavity in dogs were published between 1929 and 1932. These papers which were written by Lemon and Higgins (7–9) contain a wealth of information concerning the response of the tissues to intrapleurally introduced graphite, the channels by which this material is removed from the pleural cavities, and the sites to which it is transported. One of these papers (9) contains some observations concerning the effects of particulate matter on lymph flow from the peritoneal cavity and from the pleural cavities.

Shortly after Lemon and Higgins (7) reported their findings, Menville and Ané (10) described a roentgenographic study of the absorption of intrapleurally introduced thorium dioxide by the lym-

[1] From the Royal Edward Laurentian Hospital, Montreal, Quebec, Canada.

[2] Part of the expense of this investigation was defrayed by National Health Grants.

[3] A preliminary account of some of the experimental findings of this study was presented at the XIX International Physiological Congress, held in Montreal, Quebec, Canada, August 31–September 4, 1953. In addition, reference was made to some of these findings in a paper read at the annual meeting of the American Clinical and Climatological Association in 1953, entitled "The Pathogenesis of Tuberculosis: An Experimental Study."

phatics of the thorax and diaphragm. These investigators worked with dogs, rabbits, and rats. Their findings seem to indicate that intrapleurally introduced thorium dioxide is regularly transported, in part at least, to lymph nodes situated in sites corresponding to those to which intrapleurally introduced graphite is carried, as reported by Lemon and Higgins.

In the course of a study of the defensive mechanisms of the mediastinum, Cooray (11) injected a variety of substances, including bacteria, into the pleural cavities of rats, guinea pigs, and rabbits. He employed histologic methods to trace the routes of absorption of some of the substances with which he worked. He reported that granules of india ink regularly congregate in the retrocardiac folds; that no free ink particles were ever seen in the lungs or mediastinal tissues; and that particles were found in the paratracheal lymph nodes only occasionally. He also reported that tubercle bacilli introduced into the pleural cavities of guinea pigs are conveyed to sites corresponding to those to which ink granules are conveyed.

In their study of the absorption of fluids from the pleural spaces, Courtice and Simmonds (12) injected heparinized plasma or 0.9 per cent sodium chloride in distilled water, containing a measured amount of dye (T1824), into the left pleural cavity of cats and rabbits. At suitable intervals they collected lymph from the thoracic and the right lymphatic ducts and samples of blood from the right femoral artery for the estimation of dye concentrations. Their findings led them to draw the following conclusions: presently available evidence suggests that the dye-protein complex is absorbed as such; absorption occurs only by way of the lymphatics, much more passing through the right lymphatic duct than through the thoracic duct; the rate of absorption is greatly increased with increased lung movement; saline and plasma are absorbed at about the same rate in unanesthetized animals; and anesthesia slows down absorption.

In 1940 the present investigator (2) described the results of a series of studies in which particulate matter was introduced into the pleural spaces of guinea pigs, and roentgenographic or anatomic means, or both, were used to find the sites to which materials of this kind were conveyed. He showed that intrapleurally introduced particulate matter (colloidal thorium dioxide, finely divided lampblack, and/or tubercle bacilli) is regularly transported, in part at least, to two groups of lymph nodes, namely, the parasternal nodes and the "para-aortic" nodes. This paper, as a recent re-reading disclosed, also includes a few notes concerning the time that elapsed (at least in one experiment) between introduction of a mixture of colloidal thorium dioxide and lampblack into the pleural space and detection of one or the other, or both, of these substances in the parasternal and para-aortic lymph nodes. In the experiment just cited, each of 19 guinea pigs was given an intrapleural injection of a small quantity of the above-described mixture, all were followed roentgenographically, and selected animals were sacrificed at intervals and examined. The findings with respect to time were as follows: traces of roentgenopaque material, presumably thorium dioxide, and of lampblack were found in the parasternal nodes of 2 animals sacrificed approximately four hours after the intrapleural injection. Necropsy examination, including roentgenographic studies of portions of the sternum and of the torso with adnexa, commonly disclosed the presence of lampblack and evidence of roentgenopaque material in the para-aortic lymph nodes twenty-four to forty-eight hours following the injection. After the third day and up to the end of the second month, roentgenographic and anatomic studies regularly revealed signs of the presence of the injection mixture in both the parasternal and the para-aortic lymph nodes.

Finally, in a paper which appeared in 1954, the present investigator (3) reported one observation that had a profound effect on plans for the experimental studies which will be described in this report. The essence of this observation is that exercise in man during the acute phase of pleuritic disease, known or suspected to be of tuberculous etiology, appears to favor the development of tuberculous disease in widely separated parts of the body.

Materials and Methods

Guinea pigs were used in all of the experiments. Each animal was given a single intrapleural injection of particulate matter.

Three forms of particulate matter were employed. These were finely divided lampblack,[4] ferric oxide,[5] and colloidal iron.[6] Selected amounts of lampblack and of jeweller's rouge were dispersed in distilled water in an attempt to make suspensions of these substances: in the case of the

[4] The lampblack employed was a Grade B-Monsanto Lampblack. This preparation, according to the manufacturer, contains particles ranging in size from 0.1 to 0.4 microns.

[5] Ferric oxide (Fe_2O_3) will be described as jeweller's rouge henceforth.

[6] The colloidal iron employed was Sucrofer, an iron-sugar complex manufactured by Intramedical Products, Toronto, Ontario, Canada.

former, of 1 and of 10 per cent by weight; and in the case of the latter, of 10 per cent alone. These suspensions were placed in rubber-stoppered bottles and sterilized in an autoclave. Each bottle was shaken vigorously immediately before any material was withdrawn for intrapleural injection. Sucrofer, a preparation made expressly for intravenous administration, was used in the form in which it was supplied by the manufacturer.

The technique employed in introducing particulate matter into the pleural space was that described in a previously reported study (2). The quantities of injection mixture used ranged from 0.25 to 1.0 ml.

Immediately following the injection of particulate matter into the pleural space, all of the guinea pigs in each experiment, except as noted below, were divided into two groups. The guinea pigs in one of these groups were returned to their cages. Henceforth these animals will be referred to as control animals. The guinea pigs in the other group were forced to take exercise on one or more occasions. These will be designated as exercised animals.

Two methods were employed to force the guinea pigs to take exercise. One of these was a motor-driven treadmill to make them move at a pace equivalent to a quick walk. The other consisted of placing them in a plastic chamber and exposing them to gaseous mixtures containing various percentages of carbon dioxide and oxygen. The treadmill contained six compartments, each of which was capable of accommodating one guinea pig. Experience showed that most guinea pigs can tolerate walking at a quick pace in a treadmill for periods of twenty to twenty-five minutes. Exercise of this kind makes them breathe rapidly and deeply. The plastic chamber was large enough to hold 10 guinea pigs at one time. The gaseous mixtures to which the animals were exposed were either 5 per cent carbon dioxide, 10 per cent oxygen, and 85 per cent nitrogen; or 7 per cent carbon dioxide and 93 per cent oxygen. These mixtures were allowed to flow in and out of the chamber with a minimal degree of interference, the first mixture for approximately twenty minutes and the second for periods ranging from sixty to ninety minutes. The guinea pigs breathed rapidly and deeply while these gaseous mixtures were flowing through the chamber. No attempt was made to determine the composition of the gases in the chamber when the mixture containing 5 per cent carbon dioxide, 10 per cent oxygen, and 85 per cent nitrogen was used. The carbon dioxide content of the gases in the chamber, however, was assayed at selected periods in one experiment in which the mixture containing 7 per cent carbon dioxide and 93 per cent oxygen was employed. In this experiment the gaseous mixture was allowed to flow through the chamber at the rate of fifteen liters per minute for five minutes, and then at the rate of eight liters per minute. Examination of a sample collected seven minutes after the gaseous mixture began to flow through the chamber revealed that the percentage of carbon dioxide in the air in the chamber was the same as that in the gaseous mixture. Assays of samples collected sixteen, nineteen, forty-four, and ninety minutes following the start of the experiment disclosed concentrations of carbon dioxide of 8.1 per cent, 8.1 per cent, 9.1 per cent, and 9.8 per cent, respectively.

All of the guinea pigs, both the control and the exercised animals, were sacrificed at intervals ranging from sixteen hours to three days following the intrapleural injection of particulate matter. Most were sacrificed between sixteen and twenty-six hours following injection. A necropsy examination was carried out in each instance. Note was taken, among other things, of the presence, if any, of injection material in the tissues of the chest wall or in the lung; the quantity of fluid, if any, in the pleural spaces; and the number of parasternal and para-aortic lymph nodes, if any, which contained conspicuous deposits of particulate matter. Traces of lampblack were found in the tissues of the chest wall or in the lung on the injection side of only a few animals. No macroscopic evidence of deposits of jeweller's rouge or of colloidal iron was found in these sites. The quantities of fluid present in the pleural space on the injection side and on the contralateral side were measured in some instances. As a rule, however, the amount of fluid in the pleural spaces was estimated and recorded in terms of a trace, a moderate amount, and a large amount (table 1). Most guinea pigs appear to have six to eight parasternal lymph nodes and two para-aortic lymph nodes. These nodes, if they contain small amounts of foreign material, can be identified with the naked eye. In the experiments in which the guinea pigs were given an intrapleural injection of lampblack, it proved to be easy to count the number of parasternal and para-aortic nodes which contained conspicuous deposits of black material. Only a few of the parasternal and para-aortic nodes of these animals were removed, sectioned, and examined. Examination invariably disclosed collections of lampblack in the lymph spaces and in the parenchyma of the nodes of exercised animals. Sometimes the amount of lampblack in the sinusoids seemed completely to fill them. This was so even in nodes removed from animals which had been forced to take exercise only on one occasion. In the experiments in which jeweller's rouge or colloidal iron was introduced into the pleural space, it was not so easy to be sure of the number of nodes which contained small amounts of one or the other of these forms of particulate matter. In consequence, the nodes which were obviously larger than in healthy animals were counted, and selected ones were removed for examination. The sections of the nodes so removed were stained for iron. Examination usually disclosed either black granular material or particulate matter which gave a positive iron reaction, or both, in sections of unusually large nodes.

Results

Finely Divided Lampblack

One experiment was carried out with the control animals alone in order to determine the time required for transport of sufficient lampblack from the pleural space to the parasternal and the para-aortic lymph nodes to make most of these nodes in guinea pigs at rest appear distinctly black.

Experiment I: Fourteen guinea pigs were given an intrapleural injection of 0.5 ml. of a 1 per cent suspension of lampblack and were returned to their cages. Some of these animals were sacrificed at intervals during the first day. Others were sacrificed on the second and the third days. Necropsy examination disclosed collections of black material in the pleural spaces of all of these animals. A few of the parasternal lymph nodes of those which were sacrificed during the first twenty-four hours were black. Most of the parasternal nodes and all of the para-aortic nodes of these animals were of normal size and color. By contrast, some of the parasternal and the para-aortic nodes of most of the animals which were sacrificed on the second day, and all of the parasternal and the para-aortic nodes of all of the animals which were sacrificed on the third day, were distinctly black (figure 1).

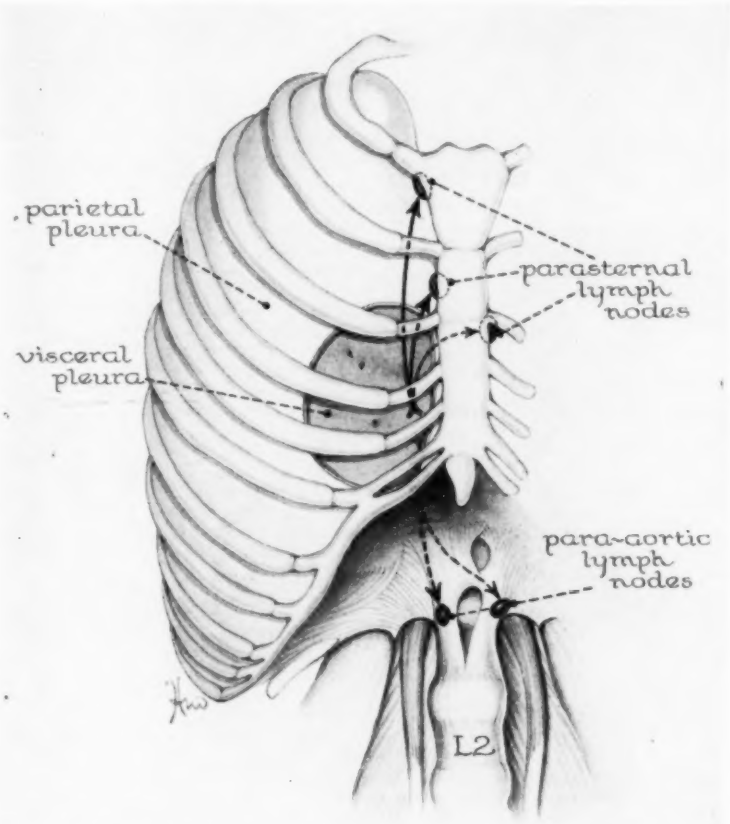

Fig. 1. Drawing illustrating the parasternal and the para-aortic lymph nodes of a guinea pig which was given an intrapleural injection of 0.5 ml. of a 1 per cent suspension of lampblack and was sacrificed at the end of the third day following the injection. Portions of three parasternal nodes and two para-aortic nodes, all of which contain black material, are plainly visible.

This experiment demonstrates that in guinea pigs given an intrapleural injection of 0.5 ml. of a 1 per cent suspension of lampblack and kept at rest, the time required to transport a sufficient quantity of this material to the parasternal and the para-aortic lymph nodes to make most of them distinctly black is approximately forty-eight to seventy-two hours. This finding appears to support the observations concerning the rate of transport of a mixture of colloidal thorium dioxide and lampblack which were recorded in the preceding section of this study.

Six experiments were carried out to permit comparison of the effects of rest and exercise on the transport of lampblack from the pleural space to the parasternal and para-aortic lymph nodes. In four (*Experiments II, III, IV,* and *V*), each guinea pig was given an intrapleural injection of 0.25 ml. of a 10 per cent suspension of lampblack. In two (*Experiments VI* and *VII*), each animal was given an injection of 0.5 ml. of a 10 per cent suspension of lampblack. One of the four experiments in which 0.25 ml. of lampblack was employed will be described below. The salient features of the other three experiments will be presented in tabular form. Thereafter the two experiments in which 0.5 ml. of lampblack was introduced into the pleural space will be described.

Experiment II: Eight guinea pigs were given intrapleural injections of 0.25 ml. of a 10 per cent suspension of finely divided lampblack. Three animals (control animals) were returned to their cages. The remaining 5 animals (exercised animals) were placed in a treadmill and forced to walk at a quick pace for twenty-five minutes immediately following the intrapleural injection. They then were returned to their cages. All of the guinea pigs were sacrificed between sixteen and eighteen hours after the lampblack was introduced into the pleural space. Necropsy examination revealed traces of black fluid in the pleural spaces of all of the control and of 4 of the exercised animals. It disclosed a considerable amount of black fluid in the pleural spaces of one of the exercised animals. Solitary black parasternal nodes were found in 2 of the 3 control animals. All of the other parasternal nodes and the para-aortic nodes of the control animals appeared to be of normal size and color. In contrast, one or more of the parasternal lymph nodes of all of the exercised animals were found to be black. Three of the 5 exercised animals had two black parasternal nodes; 2 had solitary black parasternal nodes. One of the animals that had two black parasternal nodes also had one black para-aortic node. The para-aortic nodes in the remaining 4 exercised animals appeared to be of normal size and color (figure 2).

This experiment clearly indicates that, in guinea pigs given an intrapleural injection of 0.25 ml. of a 10 per cent suspension of lampblack and sacrificed after the lapse of sixteen to eighteen hours, exercise in the form of a twenty-five minute walk at a quick pace accelerates the transport of this material to the parasternal and the para-aortic lymph nodes.

The findings in the 46 guinea pigs in the other experiments (*Experiments III, IV,* and *V*) in which 0.25 ml. of lampblack was introduced into the pleural space (table 1) support the conclusion just expressed. They also suggest that several periods of exercise by guinea pigs which have been given an intrapleural injection of lampblack favor the development of collections of fluid in the pleural spaces.

The experiments in which 0.5 ml. of a 10 per cent suspension of lampblack was introduced into the pleural space were as follows:

Experiment VI: Fifteen guinea pigs were given an intrapleural injection of 0.5 ml. of a 10 per cent suspension of finely divided lampblack. Five (control animals) were returned to their cages, 5 were exercised in a treadmill, and the remaining 5 were exposed to a gaseous mixture containing 5 per cent carbon dioxide, 10 per cent oxygen, and 85 per cent nitrogen for twenty minutes. After being given brief respites, the 5 animals which were made to walk at a quick pace and the 5 animals which were made to breathe rapidly and deeply were exercised in respectively the same manner on two other occasions. All of the animals were sacrificed approximately eighteen hours after the intrapleural injection. Necropsy examination disclosed only traces of black fluid in either or both pleural spaces of most of the guinea pigs. Quantities of fluid, about 10 ml., however, were found in the pleural spaces of 3 of the exercised animals (exposed to 5 per cent carbon dioxide, 10 per cent oxygen, and 85 per cent nitrogen three times). A few of the parasternal lymph nodes of one of the control animals were black. None of the parasternal or the para-aortic nodes of any of the other control animals contained macroscopic deposits of black material. Three of the 5 exercised animals (on the treadmill three times) had several black parasternal nodes. The parasternal nodes of the other 2 animals appeared to be of normal size and color. One or both of the para-aortic nodes of all 5 contained black material. All of the exercised animals (exposed to 5 per cent carbon dioxide, 10 per cent oxygen, and 85 per cent nitrogen three times) had several black parasternal lymph nodes. In 2 animals both para-aortic nodes were black. In the remaining 3

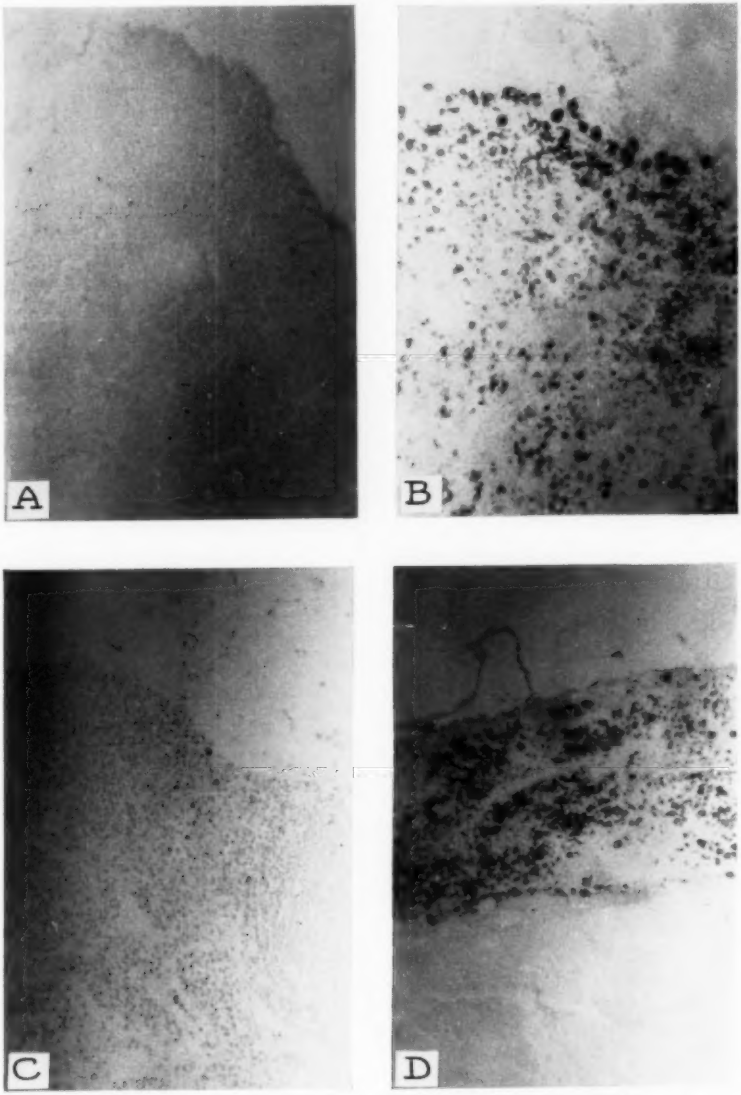

FIG. 2. Photomicrographs, taken at a magnification of 62.5 ×, of sections of the parasternal and the para-aortic lymph nodes of 4 guinea pigs which were given an intrapleural injection of 0.25 ml. of a 10 per cent suspension of lampblack and were sacrificed between sixteen and eighteen hours following the intrapleural injection. The black areas represent lampblack. The sections were stained with Nile blue to permit visualization of the tissues of the lymph nodes. (*A*) Parasternal lymph node of a control animal. (*B*) Parasternal lymph node of an exercised (treadmill once) animal. (*C*) Para-aortic lymph node of a control animal. (*D*) Para-aortic lymph node of an exercised (treadmill once) animal. Comparison of the photomicrographs of the control and of the exercised animals clearly reveals evidence of one of the effects of exercise on the transport of intrapleurally introduced lampblack.

TABLE 1

DIAGRAMMATIC REPRESENTATION OF THE AMOUNTS OF PLEURAL FLUID AND OF THE NUMBERS OF BLACK OR GRAY PARASTERNAL AND PARA-AORTIC LYMPH NODES IN 46 GUINEA PIGS

Animals by Number	1			2			3			4			5			6			7			8		
	P.S.	P.A.	Fl.	P.S.	P.A.	Fl.	P.S.	P.A.	Fl.	P.S.	P.A.	Fl.	P.S.	P.A.	Fl.	P.S.	P.A.	Fl.	P.S.	P.A.	Fl.	P.S.	P.A.	Fl.
Experiment III																								
Exercised animals (treadmill once)	●● ●● ●●	●●	++	●● ●● ●●	●●	++	●● ●●	●	++	●● ●●	●	++	●● ●	●	++	●● ●	●	++	●●	⊙	+++	●●	●	++
Control animals	●● ●●	●	++	●● ●	●	++	●●	●	++	●●	⊙	++	●●	⊙	+	●●		+	●●		+	●●		+
Experiment IV																								
Exercised animals (treadmill once)	●● ●●	●●	+++	●● ●	●●	++	●● ●	●●	++	●● ●●	●●	++	●●	●	++	●●	●	++	●●		++	●●		+
Control animals	●● ●	●	++	●●	●	++	●●	●	++	●●		++	●●	⊙	+	●●		+	●●		+	●		+
Experiment V																								
Exercised animals (treadmill three to six times)	●● ●●	●●	+++	●● ●	●●	+++	●● ●	●●	++	●● ●	●	++	●●	●	++	●●	⊙	++	●		+			
Control animals	●● ●●	●	+	●●	⊙	+	●●	⊙	+	●●		+	●●		+	●		+	●		+			

P.S.—parasternal node; P.A.—para-aortic node; Fl.—pleural fluid: + trace, ++ moderate amount (up to 10-ml.), +++ large amount. ● black node; ⊙ gray node.

animals the para-aortic node on the side on which lampblack was introduced into the pleural space was black.

Experiment VII: Four guinea pigs were given an intrapleural injection of 0.5 ml. of a 10 per cent suspension of finely divided lampblack. Two control animals were returned to their cages and 2 were exposed to a gaseous mixture containing 7 per cent carbon dioxide and 93 per cent oxygen for approximately ninety minutes.

One control and one exercised animal (exposed to 7 per cent carbon dioxide and 93 per cent oxygen once) were sacrificed three hours after the intrapleural injection. Necropsy examination disclosed that either or both pleural spaces of these animals contained traces of black fluid. The parasternal and the para-aortic lymph nodes of both animals appeared to be of normal size and color.

The remaining 2 guinea pigs, one control and one exercised animal (exposed to 7 per cent carbon dioxide and 93 per cent oxygen once), were sacrificed twenty-two hours after the intrapleural injection. Necropsy examination disclosed that the pleural spaces of both animals contained traces of black fluid. The parasternal and the para-aortic nodes of the control animal were of normal size and color. In contrast, two of the parasternal nodes and one of the para-aortic nodes of the exercised animal were jet black.

These experiments show that, in guinea pigs given an intrapleural injection of 0.5 ml. of a 10 per cent suspension of lampblack, a single period of exercise or several periods of exercise accelerate the transfer of this material from the pleural space to the parasternal and the para-aortic lymph nodes. The fact that transfer of lampblack was effected as rapidly in guinea pigs which were made to breathe rapidly and deeply (as the result of exposure to gaseous mixtures in a plastic chamber) as in the animals which were forced to walk at a quick pace in a treadmill indicates that rate and depth of respiration, or both, play important roles in the transport of particulate matter from the pleural space to the parasternal and the para-aortic lymph nodes.

Finally, it seems advisable to record that two attempts were made to acquire information concerning the roles of lampblack and of several periods of rapid and/or deep breathing in the development of moderately large to large collections of fluid in the pleural spaces of certain guinea pigs handled in the above-described manner. The findings in these experiments disclosed that lampblack or the distilled water in which it was dispersed, or both, are pleural irritants. This evidence suggests that, in the guinea pigs in which something more than traces of fluid were found, pleural irritation due to lampblack played a major role in evoking the effusions. It is recognized that other factors may have played parts in both evoking the outpouring of the fluid and delaying or preventing the removal of fluid from the pleural spaces. Some of these factors will be considered presently.

Jeweller's Rouge

One experiment was carried out to ascertain if iron, in the form of jeweller's rouge, is conveyed from the pleural space to the parasternal and the para-aortic lymph nodes and, if so, whether the effects of rest and exercise on the transport of this material are similar to such effects on the transport of intrapleurally introduced lampblack. The 15 guinea pigs which were used in this experiment were handled in a manner similar to the animals which were injected with lampblack and made to walk at a quick pace on one or more occasions (*see* table 1). Each animal was given an intrapleural injection of 0.5 ml. of a 10 per cent suspension of jeweller's rouge. Five guinea pigs (control animals) were returned to their cages. Five animals (exercised on a treadmill once) were made to walk at a quick pace for twenty minutes on one occasion; 5 animals (exercised on a treadmill three or four times), on several occasions. All of the animals were sacrificed approximately twenty-four hours after the intrapleural injection.

Necropsy examination disclosed findings that are similar to the findings in the guinea pigs which were given lampblack and forced to walk at a quick pace except that the fluid present in the pleural spaces of the control animals and of the exercised animals was a turbid yellow, and that in some instances the parasternal and the para-aortic nodes were simply abnormally large. All of the parasternal and para-aortic nodes of the control animals which appeared to be large were sectioned. Many of the parasternal and para-aortic nodes of the exercised animals also were sectioned. Microscopic examination of sections of the parasternal and the para-aortic nodes of the control animals, stained with Turnbull's blue, failed to disclose material which gave a positive iron reaction. On the other hand, examination of sections of the corresponding nodes of the exercised animals, stained in the same manner, regularly disclosed iron in particulate form. Large quantities of this material were found in

sections of the parasternal and the para-aortic lymph nodes of some of the exercised animals (on a treadmill three to four times). Examination of sections of the para-aortic lymph nodes of one of the exercised animals disclosed granules inside histiocytes which gave a positive iron reaction.

This experiment appears to leave little room for doubt that jeweller's rouge introduced into the pleural space of guinea pigs is conveyed, in part at least, to the parasternal and the para-aortic lymph nodes. It also shows that exercise, in the form of one or more short walks at a quick pace, increases the rate and the extent of the transportation of this material to their lymph nodes.

Colloidal Iron

One experiment with colloidal iron was carried out. This experiment, which among other things yielded evidence of the transport of iron from the pleural space to the parasternal and the para-aortic lymph nodes, was as follows:

Two guinea pigs were given an intrapleural injection of 1.0 ml. of colloidal iron and were made to walk at a quick pace for twenty minutes on four occasions. They were sacrificed approximately twenty-four hours after the intrapleural injection. Necropsy examination disclosed that both animals had several large parasternal and para-aortic lymph nodes. Many of these nodes were sectioned and stained: some with hematoxylin and eosin; others, with Turnbull's blue. Examination of the sections of these nodes which were stained in a routine manner disclosed coarsely granular brown pigment in the cytoplasm of phagocytes in the sinusoids. The sinusoids of the parasternal nodes contained many pigment-bearing phagocytes; the sinusoids of the para-aortic nodes contained a few. Examination of the sections which were stained for iron showed that the pigment in the phagocytes in both the parasternal and the para-aortic nodes gave a positive Turnbull's blue reaction.

The findings in this experiment clearly give added weight to the view that iron-containing particulate matter introduced into the pleural spaces of guinea pigs is regularly transported, in part at least, to the parasternal and the para-aortic lymph nodes.

Discussion

Only one investigator appears to have made an attempt to acquire information concerning the mode of spread within the pleural space of intrapleurally introduced particulate matter. Cooray (11) found that particulate matter, introduced into the pleural space of experimental animals, tends to spread rapidly to the lower mediastinal pleura and to congregate in cells in Kampmeier's foci in the retrocardiac folds. The forms of particulate matter which he employed included india ink and tubercle bacilli. No one, as far as can be ascertained, has attempted to verify Cooray's findings. It seems advisable, in consequence, to point out at this time that examination of an illustration in an account of a study by the present investigator (2) suggests that Cooray's observation with respect to the tendency of intrapleurally introduced particulate matter to spread to the lower mediastinal pleura is correct. The illustration to which reference is made contains reproductions of the chest roentgenograms of a guinea pig which was given an intrapleural injection of colloidal thorium dioxide and lampblack and was photographed on the fifth day following the injection. The reproductions (figure 2 *A*, *B*) disclose clearly defined evidence of deposits of roentgenopaque material in the lower and medial portions of the thorax.

The findings described in this communication clearly support the conclusion which the present investigator (2) reached in 1940, namely, that lampblack introduced into the pleural space of guinea pigs is regularly transported, in part at least, to the parasternal and the para-aortic lymph nodes. They also indicate that the parasternal and the para-aortic nodes of guinea pigs are the only lymph nodes to which readily detectable quantities of intrapleurally introduced lampblack are conveyed in periods up to twenty-four hours. Examination of the protocols of the guinea pigs in which lampblack was found in nodes other than the parasternal and para-aortic suggests that some of the injection material in these animals escaped into the subcutaneous tissues or was unintentionally introduced into the pulmonary parenchyma at the time of the supposed intrapleural injection. These conclusions with respect to the lymph nodes to which lampblack injected into the pleural space of guinea pigs is conveyed are similar to those reached by most investigators who have introduced particulate matter into the pleural space of experimental animals.

Certain studies, notably those of Lemon and Higgins (7–9) and of Cooray (11) appear to throw some light on the portions of the pleura through which particulate matter is removed from the pleural space and on the channels by which this

material is transported to the parasternal and the para-aortic lymph nodes. Lemon and Higgins (7) reported that the parietal pleura of dogs sacrificed several hours after an intrapleural injection of graphite regularly appears acutely inflamed, and the visceral pleura rarely exhibits signs of inflammation. They stated that, in some dogs sacrificed at this time, they found lymph channels outlined in black leading from the upper dorsocostal pleura to nodes lying near the internal mammary arteries, and still other channels which contained black material pursuing a backward course from the level of the tenth rib caudalward, traversing the diaphragm and terminating in nodes situated in the retroperitoneal space near the kidneys. Cooray (11) reported that he found little evidence of deposition of particulate matter on the visceral pleura of animals which had been given an intrapleural injection of material of this kind. These findings, along with Cooray's observations that intrapleurally introduced particulate matter tends to spread to the lower and medial portions of the thorax, suggest that the channels of small experimental animals which convey particulate matter to the parasternal and the para-aortic lymph nodes take origin in the lower mediastinal pleura or in the tissue immediately beneath this membrane. If this is the case, the lymph channels which carry particulate matter from the pleural space to the parasternal nodes probably lie entirely within the mediastinum; and those which carry particulate matter to the para-aortic nodes, partly within the mediastinum and partly within the upper stretches of the retroperitoneal region. This concept, which seemingly is completely new, appears to merit investigation.

No one seemingly has made a study of the effects of respiration on the rate and extent of removal of particulate matter from the pleural space. This is despite the fact that some evidence, as Cunningham (6) pointed out in his review of the physiology of serous membranes, suggests that respiratory movements play a part in the transport of particulate matter from serous cavities to lymph channels. The findings recorded in this communication show that a single short period of exercise or of rapid and/or deep breathing by guinea pigs increases the rate and the extent of the transportation of intrapleurally introduced lampblack and colloidal iron from the pleural space to the parasternal and the para-aortic lymph nodes. The evidence presented herein also indicates that, in this species of animal, exercise accelerates the rate at which jeweller's rouge is transported from the pleural space to these nodes. Courtice and Simmonds (12) appear to be the only investigators who have carried out experiments which have yielded findings that bear comparison with the present findings. These workers, who studied the rate of absorption of a dye-protein complex from the pleural space, found that increased lung movement in cats increases the rate at which dye-protein complex is absorbed. All available evidence indicates that Courtice and Simmonds' dye-protein complex is a large molecule. In consequence, it may be considered to be a form of particulate matter. If Courtice and Simmonds' dye-protein complex is so regarded, the fact that exercise or rapid and/or deep breathing increases the rate at which intrapleurally introduced lampblack is conveyed to the parasternal and the para-aortic lymph nodes clearly provides a measure of support for their conclusion with respect to the effects of lung movement on the rate at which intrapleurally introduced dye-protein complex is absorbed.

Only a few investigators appear to have taken an interest in the means by which fluid that bathes the pleura is formed and is removed. It seems strange that this is the case since one of the prime functions of the pleura, as the present investigator (3) pointed out in 1954, is to reduce friction as the lungs increase and decrease in size during respiration, and simple deduction suggests that the amount of secretion bathing the pleura varies from time to time depending, among other things, on the rate and depth of respiration.

These considerations and some of the findings recorded in the experimental portion of this communication already have proved to be the stimulus for studies which have brought to light a wealth of information concerning the means by which pleural fluid is formed and excess fluid is removed. A preliminary account of these studies (13) was published in 1955. Two additional papers by Burgen and Stewart (14, 15) have recently appeared in print. The findings which prompted the investigations just cited were that collections of fluid were found in the pleural spaces of many guinea pigs given a single intrapleural injection of lampblack or of jeweller's rouge; and that the number of times when the

guinea pigs were forced to take exercise appeared to bear a direct relationship to the quantities of fluid found in the pleural spaces. It will be recalled that attempts to acquire information concerning the role of lampblack in the development of moderate to large collections of fluid in the pleural spaces of certain guinea pigs disclosed that this material or the distilled water in which it was dispersed, or both, are in all probability pleural irritants.

An observation made by Lemon and Higgins (9) and the fact that the sinusoids of the lymph nodes of some guinea pigs, which had been given intrapleural injections of lampblack and which were exercised on one occasion, were found to be filled with this material, suggest that some factor, or factors, other than pleural irritation may have contributed to the development of large effusions. Lemon and Higgins found in the course of certain long-term experiments that, when lymph nodes become choked with graphite, the primary flow of particulate matter ceases. It seems possible, in the light of the foregoing, that a mechanism of the kind now described may have been responsible for the development of large effusions in certain guinea pigs handled in the manner described in this report. Lampblack or jeweller's rouge, which is transported to the parasternal and para-aortic lymph nodes at the time of the first exercise, may block the sinusoids in these nodes. As a result, fluid formed for the purpose of keeping the pleura moist during rapid and deep breathing, as well as fluid formed in response to pleural irritation, can not be removed as quickly as it is produced. Succeeding periods of exercise may have similar effects with the result that, by the end of twenty to twenty-six hours, the pleural spaces of many animals which have been forced to take exercise on several occasions contain large quantities of fluid.

Several investigators have found that particulate matter introduced into the pleural space of experimental animals may be carried to tissues or structures other than lymph nodes. Higgins and Lemon (8) reported that it is possible to recover granules of graphite from the blood stream of dogs which have been given an intrapleural injection of graphite after the anterior mediastinal nodes have become saturated with this material. They also reported that granules of graphite can be identified in the reticuloendothelial components of the liver, spleen, and bone marrow eighteen to twenty-four hours following an intrapleural injection of this material. They believe that the graphite in the liver, spleen, and bone marrow reached the blood stream by way of lymphatics to the thoracic duct. Cooray (11) reported that tubercle bacilli, which have been introduced into the pleural space of guinea pigs and phagocytized by cells in Kampmeier's foci, may be carried into the mediastinal tissues and subsequently to distant parts of the body by way of the blood stream. No attempt was made in the present study to determine the tissues or structures to which lampblack or jeweller's rouge, which had been transported from the pleural space to the parasternal and the para-aortic lymph nodes, subsequently may have been carried.

The following observations which are to be found in the present investigator's (3, 16) recent accounts of studies in the pathogenesis of tuberculosis, however, seem clearly to support Higgins and Lemon's view that particulate matter which has been conveyed to the anterior mediastinal lymph nodes may be carried to the liver and the spleen by way of the blood stream; and to give substance to Cooray's statement concerning the transport of tubercle bacilli from the pleural space to the mediastinum and from the latter to distant parts of the body. Studies in man disclosed evidence which suggests that exercise during the acute phase of pleuritic disease that is known, or suspected, to be of tuberculous etiology favors dissemination of tubercle bacilli and, in consequence, the development of extrapulmonary tuberculosis. Studies in guinea pigs, which were carried out soon after the experimental findings described in the present communication were obtained, disclosed that exercise favors the transport of tubercle bacilli from the pleural space to the parasternal and the para-aortic lymph nodes. The latter studies, a preliminary account of which appeared in a paper bearing the title "The Pathogenesis of Tuberculosis: An Experimental Study," also brought to light evidence which seems clearly to indicate that tubercle bacilli which have been transported to the parasternal and para-aortic lymph nodes may escape from these nodes and be carried in the blood stream to the liver, the spleen, the lungs, and perhaps other parts of the body.

Summary

Lampblack or jeweller's rouge introduced into the pleural space of guinea pigs is transported, in

part at least, to the parasternal and the para-aortic lymph nodes, situated in the retroperitoneal space near the attachments of the crura of the diaphragm and the points of origin of the psoas portions of the iliopsoas muscles.

The parasternal and the para-aortic lymph nodes appear to be the first—perhaps the only—lymph nodes to which particulate matter introduced into the pleural space of guinea pigs is conveyed. Evidence available at present indicates that these nodes should be considered to be the regional lymph nodes of the space between the visceral and the parietal pleura.

Some evidence suggests: that in guinea pigs the lower mediastinal pleura plays a major role in the removal of particulate matter from the pleural space; and that the lymph channels by which particulate matter is transported to the parasternal and the para-aortic nodes are situated (in the case of the former) in the mediastinum and (in the case of the latter) partly in the mediastinum and partly in the upper retroperitoneal region.

Short periods of exercise for guinea pigs, as in a motor-driven treadmill or of rapid and/or deep breathing (as in a plastic chamber through which gaseous mixtures containing either an elevated carbon dioxide and reduced oxygen content or simply an elevated carbon dioxide content are allowed to flow) increase the rate and the extent to which particulate matter is transported from the pleural space to the parasternal and the para-aortic lymph nodes.

Some observations suggest that several short periods of exercise in rapid succession favor the accumulation of fluid in the pleural spaces of guinea pigs which have been given an intrapleural injection of lampblack or of jeweller's rouge. Factors which may play a part in promoting the accumulation of fluid in the pleural spaces of animals into which particulate matter, in the forms of lampblack and jeweller's rouge, has been introduced are discussed. Attention is directed to the fact that observations made in the course of the studies described in this communication have proved to be the stimulus for other studies which have brought to light a wealth of information concerning the means by which pleural fluid is formed and excess fluid is removed.

Evidence is cited which seems to indicate clearly that particulate matter (graphite and tubercle bacilli), which has been conveyed from the pleural space to the parasternal and/or the para-aortic lymph nodes of experimental animals, may be carried to distant parts of the body, presumably by way of the blood stream.

Studies in experimentally induced tuberculous pleuritis are cited, in which advantage was taken of the findings described herein. These studies have revealed new and promising ways of tackling some of the yet unsolved problems in the pathogenesis of tuberculosis in man.

Sumario

Los Linfáticos que Drenan el Espacio Potencial entre la Pleura Visceral y la Parietal

El negro de humo o el arrebol de joyero, introducidos en el espacio pleural de los cobayos, son transportados, a lo menos en parte, a los ganglios linfáticos paraesternales y paraaórticos. Los últimos son los que están situados en el espacio retroperitoneal cerca de las inserciones de las prolongaciones del diafragma y de los puntos de origen de las porciones psoáticas de los músculos del iliopsoas.

Los ganglios paraesternales y paraaórticos parecen ser los primeros—quizás los únicos—ganglios linfáticos a los que se conducen partículas de materia introducidas en el espacio pleural del cobayo. Los datos disponibles actualmente indican que estos ganglios deben ser considerados como los ganglios linfáticos regionales del espacio que queda entre la pleura visceral y la parietal.

Algunos datos sugieren: que en los cobayos la porción inferior de la pleura mediastínica desempeña un papel principal en la eliminación de partículas de materia del espacio pleural; y que los conductos linfáticos por los que se transportan dichas partículas a los ganglios paraesternales y los paraaórticos están situados, tratándose de los primeros, en el mediastino y, en lo tocante a los últimos, en parte en el mediastino y en parte en la región retroperitoneal superior.

En los cobayos, breves períodos de ejercicio, como en un molino de rueda movido por motor o de respiración rápida y/o profunda (como en una cámara plástica por la que se hacen pasar mezclas gaseosas que contienen una proporción elevada de bióxido de carbono y reducida de oxígeno o meramente una proporción elevada de bióxido de carbono) acrecientan la velocidad y la intensidad con que se transportan partículas del espacio pleural a los ganglios linfáticos paraesternales y paraaórticos.

Sugieren algunas observaciones que varios breves períodos de ejercicio en rápida sucesión favorecen la acumulación de líquido en los espacios pleurales de los cobayos que han recibido una inyección intrapleural de negro de humo o de

arrebol de joyero. Se discuten los factores que pueden desempeñar un papel en fomentar la acumulación de líquido en los espacios pleurales de los cobayos en los que se han introducido partículas de materia, en forma de negro de humo y de arrebol de joyero. Se llama la atención sobre el hecho de que las observaciones realizadas en el transcurso de los estudios descritos en esta comunicación han servido para estimular otros estudios que ya han puesto de manifiesto un caudal de datos relativos al modo en que se forma el líquido pleural y se elimina el exceso de líquido.

Se citan datos que parecen indicar que las partículas de materia (grafito y bacilos tuberculosos), que han sido transportadas del espacio pleural a los ganglios linfáticos paraesternales y/o paraaórticos de los animales de experimentación, pueden ser llevadas a partes remotas del cuerpo, presuntamente por vía de la circulación sanguínea.

Cítanse estudios de la pleuritis tuberculosa inducida experimentalmente, en que se utilizaron los hallazgos descritos más arriba. Esos estudios han revelado nuevos y prometedores modos de abordar algunos de los problemas todavía irresueltos en relación con la patogenia de la tuberculosis en el hombre.

Resume

Les lymphatiques drainant la cavité virtuelle située entre la plèvre viscérale et pariétale

Le noir de fumée ou le "jeweller's rouge" introduit dans la cavité pleurale du cobaye est transporté, au moins en partie, jusqu'aux ganglions lymphatiques parasternaux et para-aortiques. Ces derniers sont des ganglions situés dans l'espace rétropéritonéal près de l'insertion des piliers du diaphragme et des points d'origine des faisceaux psoas des muscles psoas iliaques.

Les ganglions lymphatiques parasternaux et para-aortiques paraissent être les premiers ganglions lymphatiques—et peut-être les seuls—recevant les particules introduites dans la cavité pleurale du cobaye. Les données dont on dispose actuellement indiquent que ces ganglions doivent être considérés comme les ganglions lymphatiques régionaux de la cavité virtuelle située entre la plèvre pariétale et la plèvre viscérale.

Certains signes suggèrent que, chez le cobaye, la plèvre médiastinale inférieure joue un rôle prépondérant dans l'élimination des particules localisées dans la cavité pleurale et que les voies lymphatiques par lesquelles ces particules sont transportées aux ganglions parasternaux et para-aortiques sont situées, dans le cas des premiers, dans le médiastin, et dans le cas des derniers, en partie dans le médiastin et en partie dans la région rétropéritonéale supérieure. Chez le cobaye, de courtes périodes d'exercice, comme celui de la roue à moteur, ou par des mouvements respiratoires rapides ou profonds ou les deux, par exemple dans une chambre où circulent des mélanges gazeux contenant soit une teneur élevée en CO^2 et une teneur en oxygène réduite ou simplement une teneur élevée en CO^2, produisent une augmentation de la vitesse du transport des particules de la cavité pleurale aux ganglions lymphatiques parasternaux et para-aortiques et de la quantité transportée.

Certaines observations suggèrent que plusieurs courtes périodes d'exercice, en succession rapide, favorisent l'accumulation de liquide dans la cavité pleurale du cobaye ayant reçu une injection intrapleurale de noir de fumée ou de "jeweller's rouge". Les auteurs étudient les facteurs pouvant jouer un rôle dans l'accumulation de liquide dans la cavité pleurale des animaux à la suite de l'introduction de particules, sous la forme de noir de fumée et de "jeweller's rouge." On attire l'attention sur le fait que les observations faites au cours études décrites dans cette communication ont suscité d'autres études qui ont déjà éclairé considérablement le mécanisme contrôlant la formation du liquide pleural et son élimination, lorsqu'il est en excès. L'auteur expose les données qui paraissent indiquer nettement que les particules (graphite et bacilles tuberculeux) qui, chez l'animal en expérience, ont été transportées de la cavité pleurale aux ganglions lymphatiques parasternaux et para-aortiques, ou dans l'un des deux, peuvent être entraînées en des points éloignés, vraisemblablement par le courant sanguin.

L'auteur mentionne les études effectuées dans la pleurite tuberculeuse expérimentale, où les données précitées ont été utilisées avec profit. Ces études ont ouvert de nouvelles voies qui permettent d'espérer que l'on pourra désormais s'attaquer à certains des problèmes non encore résolus en ce qui concerne la pathogénèse de la tuberculose chez l'homme.

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THE ANTITUBERCULOUS ACTIVITY OF KANAMYCIN IN VITRO AND IN THE EXPERIMENTAL ANIMAL (GUINEA PIG)[1, 2, 3]

W. STEENKEN, JR., VINCENT MONTALBINE, AND J. R. THURSTON

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INTRODUCTION

Kanamycin was isolated from *Streptomyces kanamyceticus* by H. Umezawa and associates (1) in Japan in 1957, and was subsequently shown to have antituberculous activity upon both streptomycin-susceptible and -resistant tubercle bacilli by K. Yanagisawa and associates (2) in the same year. In the present study and in two previous ones (3, 4), the writers have confirmed Yanagisawa's findings. It has been possible to demonstrate tuberculocidal properties of kanamycin on tubercle bacilli of the H37Rv strain. It has also been possible to demonstrate that kanamycin exerts an effect on a photochromogenic strain of Mycobacteria and on tubercle bacilli resistant *in vitro* to one or more of the following drugs: streptomycin, isoniazid, neomycin, cycloserine, streptovaricin, viomycin, and para-aminosalicylic acid (PAS). In the present study, the emergence of kanamycin-resistant strains of tubercle bacilli *in vitro* is also reported, as well as the influence of the drug, when used alone and together with isoniazid or streptomycin, upon an infection induced intracardially in guinea pigs with the H37Rv strain of tubercle bacilli.

OBSERVATIONS

Effect of Kanamycin[4] on M. Tuberculosis in Vitro

Three types of liquid medium were used: Tween®-albumin, Proskauer and Beck's synthetic medium, and Proskauer and Beck's medium with 10 per cent horse serum added. The H37Rv strain and a photochromogenic strain, which were carried in Tween-albumin medium, were the test cultures. The inoculum was 0.1 ml. of a fourteen-day culture, and growth recordings were made on the seventh, fourteenth, twenty-first, and twenty-eighth days. The results are shown in table 1.

It may be noted in table 1 that, on the twenty-eighth day of growth, the H37Rv strain, when cultured in Tween-albumin medium, was resistant to 1.25 γ and susceptible to 2.5 γ of kanamycin per ml. In Proskauer and Beck's medium the organisms were resistant to 2.5 γ and susceptible to 5 γ of kanamycin per ml. The activity of the drug was only slightly diminished in 10 per cent horse serum; that is, the organisms grew in the presence of 5 γ and were inhibited in 10 γ of kanamycin per ml. The photochromogenic strain was resistant to 2.5 γ and susceptible to 5 γ of kanamycin per ml. in Proskauer and Beck medium.

Kanamycin susceptibility of seven different H37Rv strains of drug-resistant tubercle bacilli: Seven strains of tubercle bacilli resistant to viomycin, cycloserine, streptovaricin, neomycin, streptomycin,[5] PAS, and isoniazid[5] were tested in Tween-albumin medium for their susceptibility to kanamycin. Growth recordings were made on the twenty-eighth day of incubation.

All of the strains except the viomycin- and neomycin-resistant strains were resistant to 1.25 γ and susceptible to 2.5 γ of kanamycin per ml.

Cross-resistance with neomycin and viomycin: The viomycin- and neomycin-resistant strains grew in the presence of 1,000 γ of kanamycin per ml. Conversely, the kanamycin-resistant strain grew in the presence of 1,000 γ of neomycin per ml., while it grew in the presence of 1.0 γ of viomycin per ml. and was inhibited by 10 γ of the latter drug per ml.

Emergence of kanamycin-resistant tubercle bacilli: A strain of H37Rv kanamycin-resistant tubercle bacilli was produced by serial transfer in Tween-albumin medium containing increased concentrations of the drug. An inoculum of 0.1 ml. from the tube containing the highest concen-

[1] From the Trudeau Laboratory of the Trudeau Foundation, Inc., Saranac Lake, New York.

[2] This study was supported in part by a research grant (E36 C11) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service; and by Bristol Laboratories, Syracuse, New York.

[3] Presented, in part, before the Medical Session, as part of *Section 1A*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 19, 1958.

[4] Supplied by Bristol Laboratories, Syracuse, New York.

[5] The streptomycin and isoniazid were supplied by Chas. Pfizer & Co., Brooklyn, New York.

TABLE 1

IN VITRO EFFECT OF KANAMYCIN

Test Culture	Medium	Concentration	Growth in Days			
			7	14	21	28
		γ/ml.				
H37Rv kanamycin-susceptible	Tween-albumin	0	3+	4+	4+	4+
		0.04	3+	4+	4+	4+
		0.08	2½	4+	4+	4+
		0.16	1+	3+	4+	4+
		0.31	±	2+	3+	4+
		0.62	0	±	2+	3+
		1.25	0	0	1+	2+
		2.5	0	0	0	0
	Proskauer and Beck	0	3+	4+	4+	4+
		0.04	3+	4+	4+	4+
		0.08	3+	4+	4+	4+
		0.16	2½	4+	4+	4+
		0.31	2+	4+	4+	4+
		0.62	±	3+	3½	3½
		1.25	±	1+	2+	2+
		2.5	±	±	1+	1½
		5	0	0	0	±
		10	0	0	0	0
	Proskauer and Beck with 10% horse serum	0	3+	4+	4+	4+
		0.04	3+	4+	4+	4+
		0.08	3+	4+	4+	4+
		0.16	3+	4+	4+	4+
		0.31	2½	3½	4+	4+
		0.62	2+	3+	3½	3½
		1.25	1½	2½	3+	3½
		2.5	1+	1½	2+	2+
		5	0	±	1+	1+
		10	0	0	0	0
Photochromogen (acid-fast)	Proskauer and Beck	0	2½	4+	4+	4+
		0.04	2½	3½	4+	4+
		0.08	2+	3+	3½	4+
		0.16	1½	3+	3½	4+
		0.31	1½	2½	3+	3½
		0.62	1+	2+	2½	3+
		1.25	1+	2+	2½	2½
		2.5	±	1+	1½	1½
		5	0	±	±	±
		10	0	0	0	0

tration of kanamycin and showing good growth after fourteen days was used for transfer. After four transfers, the strain was resistant to kanamycin concentrations of 1,000 γ per ml. There was no loss of virulence or catalase activity of the kanamycin-resistant microorganisms.

Bactericidal action of kanamycin on tubercle bacilli: The H37Rv strain of *M. tuberculosis* was seeded in Tween-albumin medium containing the following concentrations of kanamycin: 0.4, 1.6, 6.2, 12.5, 25, 50, and 100 γ per ml. On the fourteenth day of incubation one double loopful of the culture from each tube was seeded on the surface of non-drug-containing American Trudeau Society solid medium. On the twenty-eighth day growth recordings were made and the following results were observed:

4+ growth from the 0.4 γ per ml. tube
4+ growth from the 1.6 γ per ml. tube
1+ growth from the 6.2 γ per ml. tube
44 colonies from the 12.5 γ per ml. tube
23 colonies from the 25 γ per ml. tube
40 colonies from the 50 γ per ml. tube
16 colonies from the 100 γ per ml. tube

TABLE 2

EFFECT OF KANAMYCIN, ISONIAZID, AND STREPTOMYCIN, ALONE AND IN COMBINATION, ON EXPERIMENTAL TUBERCULOSIS IN GUINEA PIGS

Groups	Drugs	Number of Animals	Average Index of Gross Tuberculosis*				
			Lungs	Liver	Spleen	Nodes	Total
1	Controls	10†	3.3	1.8	3.0	3.2	11.3
2	Kanamycin 5	10†	2.5	1.3	2.2	3.0	9.0
3	Kanamycin 10	10†	2.1	1.6	1.6	2.6	7.9
4	Kanamycin 15	5	1.2	1.2	1.2	2.5	6.1
5	Kanamycin 20	5	1.3	0.6	0.8	2.1	4.8
6	Streptomycin 10	5	1.1	0.2	0.2	2.3	3.8
7	Isoniazid 5	5	0	0	0	0	0
8	Kanamycin 5 Isoniazid 5	5	0	0	0	0	0
9	Kanamycin 10 Isoniazid 5	5	0	0	0	0	0
10	Kanamycin 15 Isoniazid 5	5	0	0	0	0	0
11	Kanamycin 20 Isoniazid 5	5	0	0	0	0	0
12	Kanamycin 5 Streptomycin 10	5	0.9	0	0.3	2.1	3.3
13	Kanamycin 10 Streptomycin 10	5	0.3	0.3	0	1.5	2.1
14	Kanamycin 15 Streptomycin 10	5	0.3	0	0.1	0	0.4
15	Kanamycin 20 Streptomycin 10	5	0.2	0	0.4	0.2	0.8
16	Kanamycin 25	10†	2.8	3.0	3.3	3.1	12.2
17	Kanamycin 50	10†	3.1	2.2	3.0	2.9	11.2
18	Kanamycin 100	10†	3.0	2.4	2.8	2.9	11.1

* Based on value of 0 to 4 for each of lungs, liver, spleen, and lymph nodes according to amount of gross tuberculous disease. Maximum was 16 for each animal.

† Died of their disease.

From these data it may be seen that, when exposed to 100 γ of kanamycin per ml. for fourteen days in Tween-albumin medium, the H37Rv strain still contains 16 viable cells per double loopful of the medium, when cultured on solid American Trudeau Society medium.

Antituberculous Activity of Kanamycin, Streptomycin, and Isoniazid, Alone and in Combination, on Experimental Tuberculosis in the Guinea Pig

One hundred and eighty guinea pigs, each weighing approximately 500 gm., were infected intracardially with 0.2 ml. of a suspension containing 0.1 mg. (dry weight) of H37Rv tubercle bacilli. The animals were divided into eighteen groups of 10 each and therapy was begun immediately. Ten animals (*Group 1*) acted as controls. The 17 remaining groups were treated for ninety days as follows:

Group 2—5 mg. (10 mg. per kg. per day) of kanamycin intramuscularly

Group 3—10 mg. (20 mg. per kg. per day) of kanamycin intramuscularly

Group 4—15 mg. (30 mg. per kg. per day) of kanamycin intramuscularly

Group 5—20 mg. (40 mg. per kg. per day) of kanamycin intramuscularly

Group 6—10 mg. (20 mg. per kg. per day) of streptomycin intramuscularly

Group 7—5 mg. (10 mg. per kg. per day) of isoniazid intramuscularly

Group 8—5 mg. and 5 mg. of kanamycin and isoniazid intramuscularly

Group 9—10 mg. and 5 mg. of kanamycin and isoniazid intramuscularly

Group 10—15 mg. and 5 mg. of kanamycin and isoniazid intramuscularly

Group 11—20 mg. and 5 mg. of kanamycin and isoniazid intramuscularly

Group 12—5 mg. and 10 mg. of kanamycin and streptomycin intramuscularly

Group 13—10 mg. and 10 mg. of kanamycin and streptomycin intramuscularly

Group 14—15 mg. and 10 mg. of kanamycin and streptomycin intramuscularly

TABLE 3

DEATHS FROM TUBERCULOSIS DURING 90 DAYS OF TREATMENT AND AVERAGE SURVIVAL TIME

Groups	Drugs	Deaths	Average Survival Time
			days
1	Controls	10/10	33
2	Kanamycin 5	10/10	46
3	Kanamycin 10	10/10	53
4	Kanamycin 15	2/10	76
5	Kanamycin 20	2/10	80
6	Streptomycin 10	0/10	90
7	Isoniazid 5	0/10	90
8	Kanamycin 5 Isoniazid 5	0/10	90
9	Kanamycin 10 Isoniazid 5	0/10	90
10	Kanamycin 15 Isoniazid 5	0/10	90
11	Kanamycin 20 Isoniazid 5	0/10	90
12	Kanamycin 5 Streptomycin 10	0/10	90
13	Kanamycin 10 Streptomycin 10	0/10	90
14	Kanamycin 15 Streptomycin 10	0/10	90
15	Kanamycin 20 Streptomycin 10	0/10	90
16	Kanamycin 25	10/10	34
17	Kanamycin 50	10/10	38
18	Kanamycin 100	10/10	39

Group 15—20 mg. and 10 mg. of kanamycin and streptomycin intramuscularly
Group 16—25 mg. (50 mg. per kg. per day) of kanamycin by mouth
Group 17—50 mg. (100 mg. per kg. per day) of kanamycin by mouth
Group 18—100 mg. (200 mg. per kg. per day) of kanamycin by mouth

At the end of ninety days of treatment, the drugs were discontinued, and the average index of tuberculosis of the animals in *Groups 1, 2, 3, 16, 17,* and *18* that had already died, as well as 5 animals that were sacrificed from each of the remaining groups, was recorded and the results may be seen in table 2. Five animals in each of *Groups 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,* and *15* were allowed to survive in order to note the rate of relapse from their infection after cessation of drug therapy, and will be reported on at a later date.

It may be observed in table 2 that *Group 1* (control animals) had an index of tuberculosis of 11.3. The animals in *Groups 16, 17, and 18,* which had received 25, 50, and 100 mg., respectively, of kanamycin by mouth, likewise died of their disease and had an index of 11.1 to 12.2. *Group 2* (5 mg. of kanamycin) and *Group 3* (10 mg. of kanamycin) had an index of 9 and 7.9, respectively. Several of the animals in these two groups died of meningitis, with a limited amount of visceral disease. This phenomenon occurs quite often when a drug is ineffective, or if the dosage is inadequate. In the remaining groups, from which 5 animals were sacrificed for comparison, it may be noted that there was no evidence of gross tuberculosis in those treated with isoniazid alone (*Group 7*) or in those animals which had a combination of kanamycin and isoniazid (*Groups 8, 9, 10, 11*).

Kanamycin is thus much less effective than isoniazid and slightly less so than streptomycin, and appears to have some additive effect when given in combination with streptomycin in dosages of 15 and 20 γ per ml.

The deaths which occurred during the ninety days of treatment and the average survival time in days are shown in table 3.

From table 3 it will be noted that the control animals had an average survival time of thirty-three days before succumbing to their disease. All of the animals in *Groups 2* (5 mg. of kanamycin) and *3* (10 mg. of kanamycin) died of tuberculosis, with an average survival time of forty-six and fifty-three days, respectively. Two animals in each of *Groups 4* (15 mg. of kanamycin) and *5* (20 mg. of kanamycin) died, with an average survival time of seventy-six and eighty days. *Groups 16* (25 mg. of kanamycin), *17* (50 mg. of kanamycin), and *18* (100 mg. of kanamycin) all died of tuberculosis, with an average survival time of thirty-four, thirty-eight, and thirty-nine days, respectively. Animals in *Groups 6* through *15*, which had received either streptomycin or isoniazid alone, or kanamycin and streptomycin, or kanamycin and isoniazid in combination, were all alive and well at the ninety-day observation period.

Toxicity

The kanamycin used in this experiment produced no noticeable toxicity in guinea pigs when used in doses of 10, 20, 30, and 40 mg. per kg. once a day intramuscularly for ninety days. Even though cross-bacterial resistance had been demon-

strated between kanamycin and neomycin, it is interesting to observe that neomycin, when used in a previous experiment (5), in a dosage of 20 mg. per kg. intramuscularly once a day, produced marked weight loss during forty-six days of therapy.

Those animals which received kanamycin in doses of 50, 100, and 200 mg. per kg. once a day by mouth showed definite signs of toxicity, which was manifested by poor appetite and loss of weight and hair. At autopsy, some of the livers were very pale in color. The drug was poorly absorbed from the intestines, as demonstrated by bioassay of the plasma. It is assumed, therefore, that kanamycin given by mouth may affect the intestinal flora.

Summary

Kanamycin inhibited the growth of the H37Rv strain of tubercle bacilli *in vitro* in concentration of 2.5 γ per ml. in Tween®-albumin medium, and in 10 γ per ml. in Proskauer and Beck medium plus 10 per cent horse serum. A photochromogenic mycobacterial strain was inhibited by 5 γ per ml. in Proskauer and Beck medium. Strains of *Mycobacterium tuberculosis* resistant to PAS, cycloserine, streptovaricin, streptomycin, and isoniazid were all susceptible to 2.5 γ of kanamycin per ml., whereas the neomycin- and viomycin-resistant strains were resistant to 1,000 γ of kanamycin per ml.

Kanamycin-resistant H37Rv tubercle bacilli grew in the presence of 1,000 γ of neomycin per ml.

Kanamycin-resistant H37Rv tubercle bacilli grew in the presence of 1.0 γ of viomycin per ml. but not in the presence of 10 γ per ml.

The drug had definite but limited bactericidal activity. The strain of *Mycobacterium* H37Rv increased in resistance quite rapidly to 1,000 γ per ml. after four transfers in Tween-albumin medium containing the drug.

Definite beneficial effect was noted in experimental tuberculosis in guinea pigs infected intracardially and treated with 30 to 40 mg. of kanamycin per kg. per day, administered intramuscularly. The drug was much less active *in vivo* than isoniazid and slightly less than streptomycin. Kanamycin may be less toxic in the guinea pig than neomycin even though it showed complete cross-resistance with the latter drug.

Sumario

La Actividad Antituberculosa de la Kanamicina in Vitro y en el Animal de Experimentación (Cobayo)

La kanamicina inhibió la proliferación de la cepa humana H37Rv *in vitro* a una concenctración de 2,5 γ por ml. en el medio de Tween®-albúmina y de 10 γ por ml. en el medio de Proskauer y Beck combinado con 10 por ciento de suero de equino. La cepa fotocromógena fué inhibida por 5 γ por ml. en el medio de Proskauer y Beck. Cepas del *Mycobacterium tuberculosis* resistentes al PAS, a la cicloserina, la la estreptovaricina, la estreptomicina y la isoniacida se mostraron invariablemente susceptibles a 2.5 γ de kanamicina por ml., en tanto que las resistentes a la neomicina y la viomicina también lo fueron a 1,000 γ de kanamicina por ml.

Bacilos tuberculosos H37Rv kanamicino-resistentes proliferaron en presencia de 1,000 γ de neomicina por ml.

Bacilos tuberculosos H37Rv kanamicino-resistentes se multiplicaron en presencia de 1.0 γ de viomicina por ml., pero no cuando había 10 γ por ml.

La droga mostró actividad bactericida bien definida, pero limitada. La cepa H37Rv del *Mycobacterium* aumentó su resistencia con bastante rapidez a 1,000 γ por ml. después de cuatro pases por medio de Tween-albúmina que contenía la droga.

Se notó efecto beneficioso bien definido en la tuberculosis experimental de los cobayos infectados intracardialmente y tratados con 30 a 40 mg. de kanamicina por kg. de peso al día, administrados por vía intramuscular. La droga fué mucho menos activa *in vivo* que la isoniacida y un poco menos que la estreptomicina. La kanamicina es mucho menos tóxica en el cobayo que la neomicina aunque desplegó resistencia cruzada con la última.

Resume

L'activité antituberculeuse de la kanamycine in vitro et chez l'animal expérimental (cobaye)

La kanamycine inhibe le développement de la souche humaine H37Rv *in vitro* à la concentration de 2,5 γ par ml. dans le milieu Tween-albumine et dans 10 γ par ml. dans le milieu de Proskauer et Beck plus 10% de sérum de cheval. La souche photochromogénique était inhibée par 5 γ par ml. dans le milieu de Proskauer et Beck. Les souches de *Mycobacterium tuberculosis* résistantes au PAS, à la cyclosérine, à la streptovaricine, à la streptomycine et à l'isoniazide étaient toutes sensibles à

2,5 γ de kanamycine par ml., alors que les souches néomycino et viomycino-résistantes étaient résistantes à 1000 γ de kanamycine par ml.

Les bacilles tuberculeux H37Rv kanamycino-résistants poussaient en présence de 1000 γ de néomycine par ml. .

Les bacilles tuberculeux H37Rv kanamycino-résistants poussaient en présence de 1 γ de viomycine par ml., et ne poussaient pas en présence de 10 γ par ml.

L'activité bactéricide de la drogue est définie mais limitée. La souche de Mycobacterium H37Rv augmentait de résistance très rapidement à 1000 γ par ml. à la suite de quatre transferts dans un milieu Tween-albumine contenant la drogue.

Un effet favorable net a été noté dans la tuberculose expérimentale de cobayes inoculés par voie intracardiaque et traités par la kanamycine intramusculaire à la dose de 30 à 40 mg par kg par jour. *In vivo* la drogue s'est montrée beaucoup moins active que l'isoniazide et légèrement moins que la streptomycine chez le cobaye. La kanamycine est considérablement moins toxique que la néomycine chez le cobaye, en dépit de l'apparition d'une résistance croisée avec cette dernière.

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OBSERVATIONS ON THE ADMINISTRATION OF KANAMYCIN TO PATIENTS WITH TUBERCULOSIS[1, 2]

KENNETH W. WRIGHT, ATTILIO D. RENZETTI, JR., JOSEPH LUNN, AND PAUL A. BUNN

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INTRODUCTION

Kanamycin is an antimicrobial drug that has recently been isolated by Umezawa and his coworkers from *Streptomyces kanamyceticus* (1). These investigators reported that the drug was effective against tubercle bacilli *in vitro* in animals and in man and, furthermore, that its inhibitory action extended to tubercle bacilli that were resistant to streptomycin (1). These *in vitro* and animal effects have been confirmed in this country by Dickison and Tisch (2) and by Steenken (3). However, tubercle bacilli resistant to viomycin and neomycin have been found to be resistant to kanamycin (4). The results of acute toxicity studies of kanamycin in animals and in man (2) warranted a more extensive trial of the drug. It is the purpose of this paper to report the observations made during the administration of kanamycin[3] for a period up to six months to patients with chronic pulmonary tuberculosis.

The kanamycin was crystallized as a sulfate salt which dissolved in water to give a mildly alkaline solution (2) and it was in this form that the drug was used in this study. It was not absorbed from the intestinal tract and hence was administered by the intramuscular route (2).

PLAN OF INVESTIGATION

Characteristics of Study Patients

Twelve patients with cavitary pulmonary disease, 11 of whom had tubercle bacilli in their sputum, were the subjects for study. Photochromogenic acid-fast bacilli were present in the sputum of the twelfth patient. With the exception of 2 patients who had been recently readmitted to the hospital, all of them had been continuously hospitalized for very long periods of time. The sex, age, extent, and duration of pulmonary disease in the group are shown in table 1. On the basis of roentgenographic interpretation, 9 of the group had predominantly chronic disease, one had predominantly acute exudative disease, and the remaining 2 had disease with elements of both types. The tubercle bacilli being excreted by all patients were resistant to streptomycin, isoniazid, and PAS. There was some variability in the kanamycin susceptibility studies made before treatment. In 8 cases, the bacilli were susceptible to kanamycin concentrations of 5 γ per ml. or less; in 2 cases, to 10 γ per ml.; and in the remaining 2, to 20 γ per ml.

Methods

In addition to the usual methods of clinical evaluation of a patient's hospital course, certain objective criteria were used to judge the therapeutic and toxic actions of kanamycin. Sputum quantity, character, and bacillary content were assessed at weekly intervals. *In vitro* drug-susceptibility tests of the tubercle bacilli isolated were performed before and during treatment. A posteroanterior roentgenogram of the chest was obtained before treatment and at monthly intervals thereafter, and laminagrams were obtained when indicated. Blood counts, hematocrit, and determinations of the erythrocyte sedimentation rate were done at monthly intervals. Urinalyses were performed at least once a week and sometimes more frequently. Determinations of the blood urea nitrogen were done at least once a month, and a phenolsulfonphthalein excretion test whenever necessary. Liver function was assessed by use of the bromsulphalein excretion test. For the purpose of detecting damage to the eighth cranial nerve, caloric and audiometric tests were done before the start of treatment and twice a month thereafter. Caloric testing was carried out by a modification of the method of Kobrak, using water of approximately 5° C. temperature (5). The concentrations of kanamycin in the blood were determined at intervals for the first twenty-four hours after intramuscular doses of 1, 0.5, and 0.25 gm. by a microbiologic assay method which employs *Bacillus subtilis* as the test organism (6). Studies of the urinary excretion of kanamycin by the

[1] From the Department of Medicine, State University of New York, Upstate Medical Center, Syracuse, New York; and the State of New York Onondaga Sanatorium, Syracuse, New York.

[2] Presented in part before the Medical Session, as part of *Section 1A*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 19, 1958.

[3] Kanamycin was supplied by Bristol Laboratories, Inc., Syracuse, New York.

TABLE 1

DISTRIBUTION OF PATIENTS BY SEX, AGE, AND DISEASE

	Number	Age	Range of Age	Extent of Disease		Duration of Disease
				Moderately Advanced	Far Advanced	
		average years	*years*			*average years*
Male.....	8	57	44–67	1	7	9
Female...	4	46	40–51	—	4	16
Total...	12	53	40–67	1	11	11

TABLE 2

DRUG REGIMEN

(Twelve Patients)

Drugs	Number of Patients
Kanamycin alone...	8
Kanamycin in combination.....	4 (3 kanamycin-PAS-isoniazid) (1 kanamycin-pyrazinimide-isoniazid)

TABLE 3

DURATION OF TREATMENT WITH KANAMYCIN

Number of Patients	Duration
5	6 months
3	5 months
2	4½ months
2	Discontinued 2 weeks 6 weeks

TABLE 4

DRUG CONCENTRATIONS IN THE BLOOD FOLLOWING INTRAMUSCULAR DOSE OF KANAMYCIN

(All Adults)

Concentrations of Drug (γ per ml.)

Hours After Dose*	1 Gram		0.5 Gram		0.25 Gram	
	Range	Average	Range	Average	Range	Average
1	9–54	30	19–50	25	5–22	10
5–8	2–17	11	2–17	10	4–11	7
12	1–9	6	0.2–12	5	1–9	4
24	0.4–3	1	0–5	0.75	0	0

* There are at least 18 observations for each of these time periods.

microbiologic assay method were carried out in 10 adults with normal renal function.

The existing treatment was continued except that streptomycin was discontinued whenever it was currently being administered. The drug regimens are indicated in table 2. The duration of treatment with kanamycin in the study subjects is noted in table 3. Although the initial 8 patients in the study were started on a dosage of 1 gm. of kanamycin per day in one intramuscular injection, the occurrence of cylindruria led to a reduction in dosage to 0.5 gm. per day at intervals varying from two to four weeks after the beginning of therapy. The remaining 4 patients were started and continued on the regimen of 0.5 gm. daily.

RESULTS

Pharmacologic Studies

The values for the kanamycin concentrations in the blood are shown in table 4 and figure 1. For each of the points in time, the values represent determinations done in at least 18 separate subjects, among whom are the 12 subjects of this study. It may be seen that there is little difference in the concentrations attained at each observation point between the 1 gm. and 0.5 gm. dose, and that concentrations above 5 γ per ml. are maintained for ten to twelve hours following either a single 1-gm. dose or multiple 0.5 gm.-doses.

The amount of kanamycin detected in the urine of the 10 normal subjects after a single dose of 0.5 gm. is shown in table 5. The total excretion of 44 per cent of the administered dose in seventy-two hours is in contrast to the 90 per cent of a total dose of streptomycin present in a twenty-four–hour specimen of urine (7).

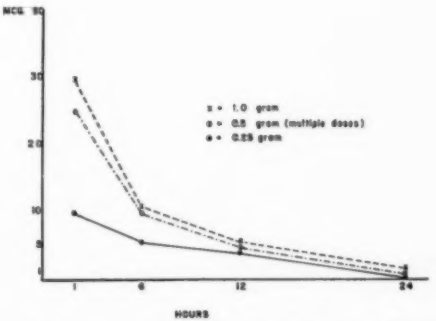

FIG. 1. Kanamycin concentrations in the blood. Each spot represents an average of at least 18 determinations in adults.

TABLE 5

URINARY EXCRETION OF KANAMYCIN
(Ten Adults with Normal Kidney Function)

Within	0.5 gm. Single Dose
(*hours*)	(*average mg.*)
8	120
16	75
24	15
48	5
72	5

Total excretion—44 per cent

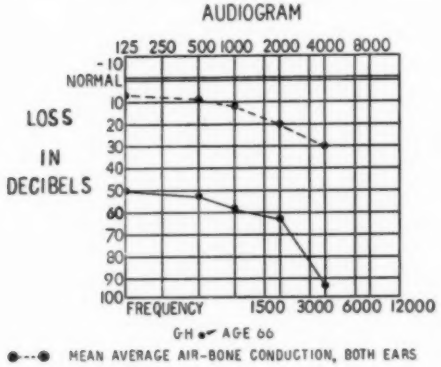

FIG. 2. Audiogram of one of the two patients who developed hearing loss involving all frequencies of both air and bone conduction after kanamycin therapy.

KM B = kanamycin therapy

DRUG TOXICITY

Renal: Granular casts appeared in the urine of all of the subjects within fourteen to twenty-eight days after the start of kanamycin treatment. The number of such casts was greater in the 8 patients who began therapy with the 1-gm. daily dose than in those who were started on the 0.5-gm. dose. The cylindruria diminished when the dose of kanamycin was reduced to 0.5 gm. per day. The blood urea nitrogen concentrations showed small elevations in 2 cases but returned to normal in spite of continued therapy. Albuminuria, hematuria, and pyuria did not occur as a result of kanamycin therapy.

Auditory: Hearing loss has developed in 2 of the 12 patients who received the kanamycin therapy. In one (figure 2), the auditory defect began in the second month of therapy and progressed to involve all frequencies of both air and bone conduction. In the other patient, the hearing loss appeared in the fourth month and was only in the higher frequencies. The total amount of kanamycin administered prior to the onset of hearing loss in these 2 cases has been approximately 35 and 48 gm., respectively.[4]

TABLE 6

PER CENT OF EOSINOPHILS DURING THERAPY WITH KANAMYCIN
(Within Three Months After Start of Therapy with Kanamycin)

	Per Cent of Eosinophils			
	0-5	6-9	10-19	20+
Number of patients...	3	3	4	2

No abnormalities of vestibular or hematopoietic function have been noted. The bromsulphalein excretion has not been impaired in any patient as a result of therapy.

Local reactions to injection of kanamycin: Although some soreness occurred at the site of injection, it did not seem any more severe than reactions to streptomycin previously experienced by each patient. No patient refused daily injection because of local discomfort.

Sensitivity phenomena: One patient developed generalized pruritis without a rash in the fourth month of treatment but this disappeared despite continuation of therapy. It may be seen from table 6 that eosinophilia was common. No other sensitivity reactions occurred.

Kanamycin resistance: The results of drug-susceptibility studies are shown in table 7. The 10 patients whose tubercle bacilli were susceptible to less than 10 γ of kanamycin per ml. before treatment are the 10 patients whose tubercle bacilli were resistant to more than 100 γ per ml. at the one hundred and twenty-day observation point; one of these 10 had photochromogenic acid-fast bacilli. The remaining 2 patients were treated

[4] Subsequent to the termination of this study, 2 additional patients of the first 12 developed some degree of hearing loss, neither as serious, however, as the original 2 described. Furthermore, observations by others indicate that eighth cranial nerve toxicity, particularly the auditory branch, is a serious problem and may become apparent after the administration of as little as 20 gm. of kanamycin (6).

TABLE 7

SUSCEPTIBILITY OF TUBERCLE BACILLI TO KANAMYCIN
(Twelve Patients)

	Number of Patients with Tubercle Bacilli Susceptible to:		
	Less than 10γ per ml.	10–50 γ per ml.	Greater than 100 γ per ml.
Before kanamycin.....	10	2*	—
At 30 days.....	8	3	1
At 60 days.....	5	5	2
At 90 days.....	2	7	3
At 120 days.....	0	2*	10

* These are the same 2 patients treated for only six and two weeks, respectively. Before kanamycin, resistance was 10 to 20.

only for six and two weeks, respectively. Of the 10 patients whose tubercle bacilli developed a high degree of resistance by one hundred and twenty days, 6 received kanamycin alone and the other 4 received it in association with antituberculous drugs to which *in vitro* resistance was already demonstrable.

Therapeutic Effectiveness

An antituberculous effect that was judged to be greater than would have been expected from bed rest and hospital care alone was demonstrated by some patients. Sputum was reduced in volume in 5 patients; in addition, 3 of these 5 showed an increased sense of well-being, gain in weight, and roentgenographic improvement, and one attained noninfectious status in the third month of therapy. Of those who improved, 3 received kanamycin alone. Two patients elected to stop the drugs, one at two weeks and the other at six weeks after the start of therapy. Both of these instances seemed to be related to the nature of the patient rather than to any specific undesirable effect of kanamycin. Of interest is the patient whose disease was associated with photochromogenic acid-fast bacilli, since roentgenographic improvement occurred after institution of therapy with kanamycin. However, within six weeks after the start of treatment his tubercle bacilli became resistant to the drug, and roentgenographic worsening was noted thereafter.

DISCUSSION

It is evident from the results reported here that the intramuscular administration of 0.5 to 1 gm. of kanamycin to tuberculous patients led to drug concentrations in the blood which inhibited the growth *in vitro* of the strains of tubercle bacilli isolated from 11 subjects. The drug concentrations attained likewise inhibited the growth of a photochromogenic acid-fast bacillus from a patient with pulmonary disease. As 10 to 20 γ of kanamycin per ml. were required to inhibit the growth of tubercle bacilli in 2 of the 11 tuberculous subjects and as the blood concentration of drug attained with daily doses of 0.5 gm. was in no instance less than 19 γ per ml., it seems advisable to use single injections of no less than 0.5 gm. of the drug in the treatment of tuberculosis. It is also apparent that little is to be gained by administering 1 gm. of the drug since the curve of blood concentration following this dose does not differ significantly from that following 0.5 gm. As with previous antituberculous drugs, the interval between doses will have to be determined by other factors as well as by the concentrations of drug attained in the blood.

The mechanism of the hearing loss in 2 patients is not apparent. Neither age nor total dosage of drug administered are likely to be the sole factors involved in this form of drug toxicity since other patients in the same general age group have received larger doses of the drug. The finding of granular casts in the urine of all patients is probably not on the basis of impurities, since kanamycin is said to be a crystalline material of a high degree of purity (2). Further observation is required to determine whether more serious renal toxicity will result from continued use of the drug.

The frequent occurrence of eosinophilia suggests that hypersensitivity reactions may be anticipated with kanamycin.

The rapid appearance of strains of tubercle bacilli highly resistant to kanamycin makes it obvious that, if prolonged usage is contemplated, attempts will have to be made to delay this resistance by the concomitant use of another antituberculous agent.

Judgment of the therapeutic efficacy of kanamycin in the series of cases of chronic pulmonary tuberculosis reported here is difficult since the type of disease treated was of a variety in which dramatic changes could not be expected. Nevertheless, the disappearance of tubercle bacilli from the sputum in one case and the favorable changes in sputum volume, body weight, and the chest roentgenograms in a few patients indicate that the drug is sufficiently effective to warrant further

trial. On the basis of this study of a small number of patients, it seems that kanamycin has some antituberculous activity in the human.

Summary

Kanamycin, a new antimicrobial which is effective in low concentrations *in vitro* against tubercle bacilli, has been administered to 11 subjects with chronic pulmonary tuberculosis for periods up to six months. With a daily dose of 0.5 gm., blood concentrations above the usual inhibitory concentrations were achieved and were maintained for eight hours. Two patients who were treated initially with 1 gm. of kanamycin daily developed diminution in hearing. Granular casts in the urine appeared in all patients. No more serious renal toxicity occurred nor was there evidence of toxicity to the liver, vestibular apparatus, or hematopoietic system. Other than eosinophilia, which was common, no sensitivity phenomena of significance were observed. Bacterial resistance was present at sixty days in half of the patients and at one hundred and twenty days in all. Clinical and roentgenographic evaluation indicated that kanamycin had some antituberculous activity in the human and that, in combination with another antituberculous agent, it should be given a trial in a larger series of patients.

Of additional interest was the observation that kanamycin inhibited the growth of a strain of photochromogenic acid-fast bacilli. This finding suggests that the drug also be given further trial in the treatment of diseases associated with atypical acid-fast bacilli.

Sumario

Observaciones sobre la Administración de Kanamicina a los Tuberculosos

A 11 sujetos que habían tenido tuberculosis pulmonar crónica por períodos hasta de seis meses se les administró kanamicina, nuevo antimicrobiano que es eficaz a concentraciones bajas *in vitro* contra los bacilos tuberculosos. Con una dosis diaria de 0.5 gm., se obtuvieron y mantuvieron durante ocho horas concentraciones sanguíneas superiores a las habituales concentraciones inhibidoras. Dos enfermos tratados inicialmente con 1 gm. diario de kanamicina manifestaron disminución de la audición. En todos los enfermos aparecieron cilindros granulares en la orina. No hubo signos de toxicidad renal más grave ni tampoco de toxicidad para el hígado, el aparato vestibular o el sistema hematopoyético. Aparte de eosinofilia, que fué común, no se observaron fenómenos de susceptibilidad de mayor importancia. A los sesenta días había resistencia bacteriana en la mitad de los enfermos y a los ciento y veinte días en todos. La justipreciación clínica y radiográfica indicó que la kanamicina poseía alguna actividad antituberculosa en el hombre y que, combinada con otro medicamento antituberculoso, debe ser objeto de una prueba en una serie más numerosa de enfermos.

Nuevo interés motivó la observación de que la kanamicina inhibía la proliferación de una cepa de bacilos ácidorresistentes fotocromógenos. Sugiere este hallazgo que la droga debe ser además más ensayada en el tratamiento de enfermedades relacionadas con bacilos ácidorresistentes atípicos.

Resume

Observations sur l'administration de la kanamycine aux tuberculeux

La kanamycine est un nouvel antimicrobien, efficace à faible concentration contre le BK *in vitro*, qui a été administré pendant des périodes atteignant six mois à onze sujets atteints de tuberculose chronique. Une dose quotidienne de 0,50 g a permis d'obtenir et de maintenir pendant huit heures des concentrations sanguines supérieures aux concentrations inhibitrices usuelles. Deux malades, qui furent initialement traités avec 1 g de kanamycine par jour, ont présenté une diminution de l'audition. L'urine de tous les malades contenait des cylindres granuleux rénaux. Il n'y a eu aucun autre signe de toxicité rénale plus grave. On n'a observé aucune preuve de toxicité au niveau du foie, du système vestibulaire ou hématopoïétique. En dehors de l'éosinophilie, qui était habituelle, aucun phénomène appréciable d'intolérance n'a été observé. Chez la moitié des patients la résistance bactérienne existait au soixantième jour, et chez tous au bout de cent-vingt jours. L'évaluation clinique et radiologique a révélé que la kanamycine possède une certaine activité antituberculeuse chez l'homme et qu'un essai de la kanamycine en association avec un autre agent antituberculeux devrait être fait chez un groupe de malades plus important.

L'observation de l'inhibition de la croissance d'une souche de bacilles photochromogéniques acido-résistants par la kanamycine présente un intérêt additionnel. Ce résultat suggère que l'on doit poursuivre l'expérimentation de la drogue dans le traitement des affections dans lesquelles des bacilles acido-résistants sont intéressés.

Acknowledgments

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Case Reports

"IMMEDIATE" TYPE OF COCCIDIOIDIN SKIN REACTION IN PULMONARY COCCIDIOIDOMYCOSIS[1]

ADRIAN G. KRUDY[2] AND RICHARD HOFF

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Introduction

The coccidioidin skin reaction usually is of the "delayed" type and similar to that of tuberculin. However, in rare instances, an "immediate" type of reaction is also encountered. Hirsch and associates (1, 2) found that skin tests with filtrate of liquid cultures of *Coccidioides immitis* in guinea pigs infected experimentally and in a 9-year-old Negro girl with chronic disseminated coccidioidomycosis have produced reactions of two kinds: (*a*) an immediate reaction, in the form of a wheal occurring within a few minutes and subsiding considerably in one hour, and (*b*) a delayed reaction, noted as a redness and swelling of the skin, reaching its maximum in twenty-four to thirty-six hours.

Smith and associates (3) also observed "immediate" reactions in persons who failed to develop a "delayed" type of reaction and "who were too recently in the endemic area to have acquired infection and lost sensitivity." However, they claim to have elicited similar reactions with sterile water. In two "very sensitive" persons they have seen generalized urticaria develop eight to eighteen hours after the skin test. One of these patients was still undergoing his infection, and the other showed no evidence of current infection.

The case presented is that of a culturally proved pulmonary coccidioidomycosis with a marked "immediate" but with a negative "delayed" type of coccidioidin skin reaction.

Case Report

A 25-year-old white male was admitted to the hospital on December 16, 1957, because of hemoptysis and a weight loss of 28 pounds. Within the past few years he had visited in Mexico and in Texas.

The physical examination at the time of admission was unremarkable. Roentgenographic studies of the chest revealed a 2.5 cm. thin-walled cavity in the right upper lobe. Repeated examinations of the sputum and gastric contents were negative for acid-fast organisms.

The routine skin tests were applied, consisting of intermediate-strength PPD (0.0001 mg.), histoplasmin (1:100), blastomycin (1:100), and coccidioidin (1:100)[3] intradermally. All of these were read as negative at forty-eight to seventy-two hours.

Because of the suspicion that the patient had a coccidioidal cavity, the coccidioidin skin test was repeated in a 1:10 dilution. The patient was seen within fifteen minutes and an immediate type of wheal and erythema was noted which involved almost the entire forearm. The reaction completely disappeared within two hours and no delayed reaction was observed at forty-eight hours. At this point, the patient volunteered the information that a similar reaction had occurred with the earlier test dose (1:100 dilution).

Subsequently resectional surgery was performed which revealed the organism of *Coccidioides immitis* in histologic section, and culture of the surgical specimen also yielded these organisms.

Comment

In the past two years, the writers have observed the skin reactions in about 1,000 patients to tuberculin, histoplasmin, blastomycin, and coccidioidin without encountering an "immediate" type of reaction except in the case reported herein. It is to be noted that repeated usage of coccidioidin does not evoke antibodies (3) and, although a sterile water control injection was not used in this case, the patient did not react to tuberculin, histoplasmin, or blastomycin.

Considering the rarity of this type of reaction, it is believed that for routine purposes a reading

[1] From the Pulmonary Disease Service, Valley Forge Army Hospital, Phoenixville, Pennsylvania.
[2] Present address: Veterans Administration Hospital, 7300 York Road, Cleveland 30, Ohio.

[3] The coccidiodin used was manufactured by Cutter Laboratories, Berkeley, California, and consists of a 1:10 dilution of the filtrate obtained from a culture of 10 selected strains of *Coccidioides immitis*. The 1:100 dilution was prepared with sterile physiologic saline.

of coccidioidin skin tests at forty-eight hours is satisfactory.

Summary

A case of an "immediate" coccidioidin skin reaction with 1:100 and 1:10 dilutions is reported in a 25-year-old white male who had a thin-walled pulmonary cavity. The "delayed" type of skin reaction at forty-eight hours was negative. Resection of the pulmonary lesion was performed and *Coccidioides immitis* was demonstrated in sections and was also recovered by culture.

Sumario

Cutirreacción de Forma "Inmediata" a la Coccidioidina en la Coccidioidomicosis Pulmonar

Preséntase un caso de cutirreacción "inmediata" a la cocciodioidina a diluciones al 1:100 y 1:10 en un varón blanco de 25 años de edad que tenía una caverna pulmonar de paredes delgadas. La forma "retardada" de cutirreacción a las cuarenta y ocho horas resultó negativa. Se ejecutó la resección de la lesión pulmonar y se descubrió el *Coccidioides immitis* en los cortes, obteniéndose también por cultivo.

Resume

Réaction cutanée à la coccidioïdine de type "immédiat" dans la coccidioïdomycose pulmonaire

Description d'un cas de réaction cutanée à la coccidioidine de type "immédiat" avec des dilutions à 1/100 et à 1/10 chez un blanc de 25 ans qui présentait une caverne pulmonaire à paroi mince. La réaction cutanée de type "retard" à la quarante-huitième heure était négative. La résection de la lésion pulmonaire a été effectuée et *Coccidioides immitis* a été isolé dans les coupes et recouvré en culture.

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Editorial

Progress Toward the Standardization of BCG Vaccine

Incontrovertible evidence is now available that, when properly administered, BCG vaccination can decrease the incidence of pulmonary tuberculosis in human beings. Among recent examples of successful use of the vaccine under practical conditions, one need only mention the well-documented account by T. V. Hyge of an outbreak of tuberculosis in a Danish girls' school and the statistical study carried out among school leavers in England by the Medical Research Council. On the other hand, it must be recognized that there are also many records of failure—a fact which points to the existence of important unknowns in the practice of vaccination. One of these unknowns which is hardly ever considered is the quality of the sample of vaccine used in a particular test. For it can no longer be doubted that the label BCG covers products exhibiting gross differences—qualitative as well as quantitative—in protective effectiveness.

Fortunately, much useful information relevant to this problem has been gained during recent years from studies on BCG vaccines subjected to comparative tests *in vitro*, in experimental animals, and in human beings.[1] As this information suggests that techniques can be devised to evaluate the immunizing effectiveness of BCG preparations, it appears worth while to review briefly at this time the knowledge which might serve as a rational basis for standardization.

* * *

Among the difficulties which stand in the way of standardization of the vaccine, the first to be considered is the genetic variability of the BCG strain. As is well known, all cultures of BCG are derived from the attenuated strain of bovine tubercle bacilli first isolated by Calmette and Guérin at the Pasteur Institute. At first sight, identity of origin would seem to assure uniformity of characteristics, but this is far from the truth. Like all other bacterial strains, BCG continuously undergoes mutations. Since no attempt has been made to select a particular form among these countless mutants, it is not surprising that the cultures now used in the various vaccine-production laboratories differ profoundly one from the other. These differences among the substrains manifest themselves in colonial morphology, rate of growth, and other biochemical activities. Of special importance is the fact that the substrains used for vaccine production differ markedly in their behavior *in vivo*.

When injected into guinea pigs or into mice, the various substrains of BCG differ in the extent of their multiplication and persistence in the various organs, as well as in the severity and duration of the lesions that they produce. In man also, there is suggestive evidence that the results of vaccination differ with the BCG substrains used for making the vaccine, as manifested by the duration of tuberculin allergy and the incidence of adenopathies.

The physiologic state of the bacilli also plays a part in the variability of the vaccine. In this regard, one of the significant variables is the age of the culture at the time of its harvest for the preparation of the vaccine. This factor is of extreme importance because—as could be expected from classic knowledge of bacterial physiology—the metabolic activity of BCG and its behavior *in vivo* are profoundly affected by the age of the cells. Thus, young BCG cells possess much greater invasive power in experimental animals than do older cells, and there is good evidence that they are also more active in human beings.

The length of time the vaccine has been kept after preparation and the conditions of storage (including such factors as temperature, composition of the suspending fluid, and perhaps exposure

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[1] This knowledge has been well described and appraised in a very timely international symposium which was held in Paris from September 22 to September 26 under the joint auspices of the International Union Against Tuberculosis and of the International Children's Centre. Professor Armand Frappier, Director of the Institut de Microbiologie et d'Hygiene in Montreal, organized the symposium and carefully focused the discussions on the study of techniques that could possibly be used for the evaluation of BCG vaccine.

A summarized account of the symposium will be presented by Dr. W. Kurylowicz in a forthcoming issue of the *Bulletin of the International Union Against Tuberculosis*. Moreover, the full proceedings will be eventually published in the form of a monograph by the International Children's Centre. There is no need therefore to report here discussions that took place at the symposium. In the present essay, I have attempted to outline some of the facts on which there appeared to exist a large measure of agreement. But I wish to emphasize that the symposium ended without formulating recommendations, and that the views expressed here are purely personal.

to light) constitute other physiologic determinants which affect the activity of BCG. Only scattered observations have been made concerning the influence of these factors on the protective activity of the vaccine. One finding, however, appears of general significance, namely, the direct relation which exists between the numbers of viable BCG cells injected and the intensity of the response elicited *in vivo*. This fact is of enormous practical importance because the number of viable cells in the vaccine decreases rapidly when the latter is placed under inimical conditions, especially when the temperature of storage is somewhat elevated. There is no doubt that different samples of vaccine bearing the same batch number can differ profoundly in viable bacterial population, and therefore in biologic activity, unless they have all been packaged and stored under identical conditions.

* * *

Granted the complexity of the factors which affect the behavior of BCG *in vivo*, it appears nevertheless that general methods can be formulated for an evaluation of the activity of the vaccine. Some of these methods are based on the assumption that the protective activity of BCG is an expression of its ability to multiply *in vivo*. In fact, it has been shown that batches of vaccine differ greatly one from the other with regard to the fate of the BCG bacilli injected into experimental animals, and to the pathologic effects that they produce. The extent of bacillary multiplication in the organs of mice following injection of BCG by various routes (intraperitoneal, intravenous, air-borne) and the type of local reaction produced in guinea pigs by intradermal injection of graded doses of vaccine are two manifestations of BCG which lend themselves to quantitative measurement.

The test most commonly used for evaluating the activity of BCG vaccine is its ability to elicit tuberculin allergy in the guinea pig and in man. As usually practiced, however, this test cannot be regarded as a technique for measuring protective activity. First is the fact that the allergizing power and the ability to elicit immunity are two independent characteristics which do not necessarily coincide so that the former cannot be used as an index of the latter. Furthermore, even limiting the discussion to the allergizing power of the vaccine, the tests as commonly carried out are far from adequate. The only information usually provided concerning activity of the vaccine is that injection of a given dose, either in man or in guinea pigs, converts most of the vaccinated individuals to the tuberculin positive state within a few weeks or months. But it is well established that vaccines differ greatly as to the minimal amount required to produce allergy, and even more as to the duration of the allergy thus produced.

Ideally, a titration of allergizing activity would demand a comparison of the effect of several doses of vaccine, with special regard to the duration of allergy. These tests cannot be made in human beings, but they can be made in experimental animals. It is an encouraging fact in this respect that it appears possible to predict the duration of the allergy in human beings from the qualitative character that it manifests when the first tuberculin test is done shortly after vaccination.

Whereas such an enormous amount of work and talk is devoted to the ability of BCG vaccine to elicit tuberculin allergy, there is surprisingly little emphasis on its ability to protect against infection. Yet, the fact that tuberculin allergy cannot serve as a measure of immunity to tuberculosis makes it imperative to evaluate BCG vaccine by tests measuring protective activity.

Fortunately several kinds of protective tests in experimental animals can be used for this purpose. Comparison between the survival time of vaccinated and of control animals infected with virulent bacilli is of course the simplest way to measure protection, but this technique is most unsatisfactory for theoretic and practical reasons that cannot be discussed here. Evaluation of the extent of disease by the study of lesions has been widely used, especially in guinea pigs. Of special interest in this regard is the fact that vaccination reduces considerably the severity of the lesions produced in guinea pigs by the intradermal injection of very small doses of virulent bacilli.

Quantitative bacteriologic studies have also been made of the restrictive effects of vaccination on the multiplication of virulent bacilli in the organs of guinea pigs and mice. These studies, based on the microscopic enumeration of bacilli in stained sections, and on the determination of the numbers of viable bacilli in various organs by cultivation on appropriate culture media, have provided very striking evidence of the protective effect of BCG vaccination.

Needless to say, tuberculosis in man has pathologic characteristics that differentiate it from the experimental disease in the guinea pig and the mouse. Nevertheless, it can be surmised from theoretic immunology that the protective mechanisms which limit bacterial multiplication in experimental animals also operate in the human disease.

* * *

In summary, any attempt at standardization of BCG must take cognizance of the fact that several

independent factors can affect the protective activity of the vaccine. In the present state of knowledge, the best defined of these factors are: (*a*) the intrinsic biologic characters of the substrain of BCG employed for preparing the vaccine; (*b*) the physiologic state of the cells in the sample of vaccine—in particular as affected by the age of the culture at the time of harvest and by the conditions of storage; (*c*) the numbers of viable cells in the dose of vaccine injected.

At the present time, no serious attempt is made to assay these parameters in the vaccines distributed for human use. At the most, some tests are made to confirm that the vaccine can produce tuberculin allergy. But, as carried out in practice, these allergy tests have little quantitative significance. Furthermore, it is almost certain that tuberculin allergy is independent of immunity to infection and that measurement of the former does not provide convincing information concerning the protective activity of the vaccine.

Thus, it is probable that many of the divergencies in the results of vaccination arise from the fact that the label BCG covers products exhibiting gross differences—qualitative as well as quantitative—in biologic activity. Furthermore, the margin of safety in the dose of vaccine that can be effectively used is far smaller than is commonly believed. As is well known, increasing the dose by a few fold commonly results in a disturbing frequence of adenopathies. On the other hand, decreasing the dose by a few fold may sharply reduce the percentage of successful "takes"—at least as measured by the percentage and duration of allergy. In view of these facts, there is a crying need for criteria defining the significant characteristics of the vaccine and, in particular, for quantitative techniques to measure its protective activity.

The present essay is not concerned with the indications for, or objections to, the use of BCG vaccine. For this reason, there is no need to review here the well known epidemiologic considerations which limit the advisability of vaccination. But it seems proper to emphasize that, when BCG is used, more care should be exercised in controlling the protective activity of the vaccine. Because of the urgency of the tuberculosis problem in many parts of the world, it was permissible a decade ago to engage on huge vaccination programs with vaccines of untested potency. The time has now come, however, when the international and national organizations which finance and operate BCG programs should recognize as one of their responsibilities the formulation of strict criteria to assure the protective effectiveness of the vaccine. These criteria must include not only the numbers of BCG cells to be injected but also the ability of these cells to increase resistance to tuberculosis.

René J. Dubos
New York, New York

Notes

AN URBAN FOCUS OF HISTOPLASMIN SENSITIVITY

In the spring of 1957 most of the 2,400 high school students in Whitfield and Murray Counties, Georgia, were photofluorographed and skin tested with histoplasmin, coccidioidin, and tuberculin. As reported elsewhere,[1] a few reacted to coccidioidin; approximately 5 per cent reacted to tuberculin (5 TU); and results with histoplasmin revealed twice as many reactors among students living in the city of Dalton as in the surrounding rural areas: 55 per cent compared with a little more than 20 per cent. To obtain more information on the pronounced geographic differences in histoplasmin reactor rates, especially because of the reversal of the usual urban-rural picture, a second survey similar to the first was made of elementary school pupils in the fall of 1957.

Both the spring and fall programs were carried out cooperatively by the Dalton-Whitfield County Health Department and the Tuberculosis Program of the Public Health Service. Arrangements for the programs were made by Cecil F. Jacobs, District Director, and Dorothy Barfield, Nursing Supervisor, of the Health Department. The testing and reading were done by a Public Health Service field research team, Birthe Johansen reading almost all of the reactions.

Whitfield and Murray Counties are adjacent counties in the northwestern part of Georgia. They both abut the Tennessee border on the north; Whitfield County is approximately fifty miles east of the Alabama border, Murray County is just east of Whitfield. Together the two counties cover an area of 600 square miles and have a population of 53,000, of whom 19,000 live in Dalton, Whitfield's county seat, and 2,500 in Chatsworth, Murray's county seat. Aside from the two urban centers, the remaining areas of both counties are essentially rural. The economy of the area is closely tied to the tufted textile industry (chenille and other products). Much of the rural population and most of the urban population work in the textile mills. Farming is no longer of importance except for the recently developed chicken industry.

The 23 elementary schools in both counties, with a total enrollment of 9,400, were included in the fall testing program. Histoplasmin H-42 in a dilution of 1:50 was injected intradermally in 0.1 ml. Tests were also given with 1:100 coccidioidin and 5 TU of tuberculin. Reactions were read on either the second or third day; the diameter of induration was measured and recorded in millimeters.

At the time of the testing a residence history was taken of each child. Among the youngest, the histories were often incomplete. All elementary school children who were born in either Whitfield or Murray County and who gave no indication that they had ever lived elsewhere were later classified as "lifetime" residents. Only material for lifetime residents will be presented here.

Supplementary information about possible sources of exposure to the fungus *Histoplasma capsulatum* was obtained from questionnaires submitted to two groups of Dalton residents. The students at Dalton high school were asked to answer questions about time spent on farms, fishing and hunting trips to Tennessee, household animals (including chickens), and fertilizers used on their lawns or gardens. Information was also obtained from a randomly selected sample of 300 households of "lifetime resident" elementary school pupils in connection with a sanitary survey of Dalton in April, 1958, conducted under the direction of Richard Clapp of the Public Health Service Communicable Disease Center. The respondent in each household in the sample was asked specific questions about the use of fertilizer on the lawn or garden in addition to inquiries about (and inspection for) animals of any kind kept on the premises. Soil specimens were also taken from various places around each house and sent to Libero Ajello at the Communicable Disease Center, who kindly consented to make soil cultures for isolation of the fungus.

Results of the histoplasmin tests are presented for all elementary and high school students classified as lifetime residents—approximately half of the total numbers tested. The few Negro students have been excluded. Because summer vacation intervened between the two testing programs, pupils who finished the eighth grade in June and moved into high school in the fall were not tested in either program. Analysis of frequency distributions of the sizes of reactions to histoplasmin, and of the relationship between photofluorographic evidence of pulmonary calcification and size of reaction in the high school students, supports the interpretation of reactions measuring 6 or more mm. of induration as positive and those measuring less than 6 mm. as negative for histoplasmal infection in this area.[1, 2]

The percentage of reactors increases steadily,

[1] Edwards, P. Q., *et al.*: Dis. Chest, 1958, *34*, 467.

[2] Palmer, C. E., and Edwards, P. Q.: Am. Rev. Tubere., 1958, *77*, 546.

with no difference between boys and girls, up to the age of twelve years. Over the age of twelve, however, nonreactors become reactors at a slightly faster rate among boys than girls. Age curves are given in figure 1 of reactors for children attending schools in the two urban districts, Dalton and Chatsworth, and in the rest of Whitfield and Murray Counties. With few exceptions, children living in Dalton or Chatsworth attend school in the city while all others attend the county school located nearest to their homes. The percentage of reactors in each school, therefore, gives a fair estimate of the prevalence of sensitivity in the vicinity.

Dalton residents, as shown in figure 1 and also in table 1, have the highest frequency of histoplasmin reactors, increasing from 14 per cent at the age of six to seven years to 60 per cent at eighteen to nineteen years. In Chatsworth, which is 14 miles east of Dalton, there were no reactors up to the age of ten and only 16 reactors among 201 children between ten and nineteen years of age. For the rural parts of both counties, the rates are intermediate between the two urban areas and differ but little from school to school, except for somewhat higher rates in Tunnel Hill and Valley Point. The steady increase in all age curves suggests that the infective agent has been present for many years, an assumption supported by the frequency of pulmonary calcifications in tuberculin-negative, histoplasmin-positive high school students.[1, 2]

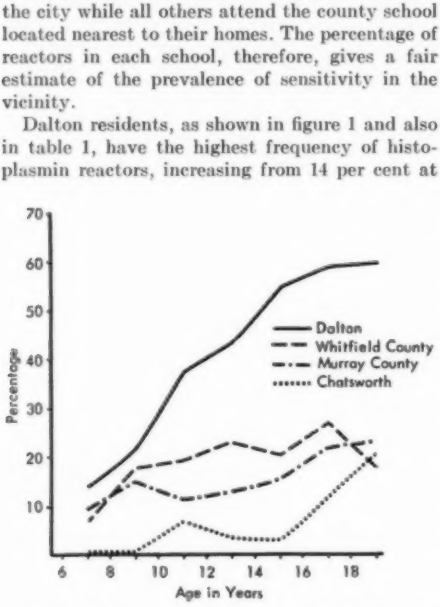

FIG. 1. Percentages of reactors to histoplasmin among school children, by age and by location of residence.

Because of the high reactor rate within Dalton, a large street map of the city was obtained and the home of each child was located. (Approximately 100 children attending school in Dalton live outside the city limits and were not included.) A red *X* was placed on the map for each child who had a reaction, a yellow *X* for each nonreactor. A clear pattern emerged, showing a high concentration of reactors in the western section and what appeared to be a random scattering of reactors throughout the rest of the city. Boundaries were arbitrarily set around the high prevalence area by drawing straight lines on the map.

Figure 2 is an outline map of Dalton. The business district lies along the railroad tracks which run in a valley through the center of town. The older homes are situated around the business district with the newer residential areas in the more outlying districts on both sides of town. The topography of Dalton is best described as hilly, with pine and oak the predominant native trees.

The section with high histoplasmin reactor rates, identified as Section *A* on the map, is an affluent residential section with both old and new

TABLE 1

NUMBER OF SUBJECTS TESTED WITH HISTOPLASMIN AND NUMBER WITH REACTIONS OF 6 OR MORE MM. AMONG LIFETIME RESIDENTS OF WHITFIELD AND MURRAY COUNTIES, GEORGIA, 1957

Age in Years	Dalton Section A		Dalton Section B		Whitfield County*		Chatsworth		Murray County†	
	Number Tested	Number ≧ 6 mm.	Number Tested	Number ≧ 6 mm.	Number Tested	Number ≧ 6 mm.	Number Tested	Number ≧ 6 mm.	Number Tested	Number ≧ 6 mm.
6–7	67	16	174	18	320	22	61	—	84	8
8–9	59	31	265	39	437	79	38	—	93	14
10–11	56	42	204	56	510	98	45	3	87	10
12–13	36	30	178	63	335	77	56	2	62	8
14–15	21	18	85	40	364	73	33	1	91	14
16–17	30	27	99	49	351	94	42	5	114	25
18–19	11	9	56	31	139	25	25	5	65	15
Total	280	173	1061	296	2456	468	300	16	596	94

* Excluding Dalton
† Excluding Chatsworth

homes. One is impressed in walking through the area by the lush appearance of the lawns and the well-kept gardens. All of the streets in this section are paved.

Section *B* is a heterogeneous area containing the textile mills and the less affluent residential areas, but several districts have new homes similar to those in Section *A*. The lawns and gardens in some parts of Section *B* are not impressive; and many of the streets are unpaved.

Age curves of reactors to histoplasmin are given separately in figure 3 (*see* also table 1) for children living in Sections *A* and *B* of Dalton. The percentages in Section *A* begin at 24 for children six to seven years of age and rise to more than 80 by fifteen years. From these results it can be estimated that infection with Histoplasma occurs at a rate of 10 to 15 per cent per year, which is considerably higher than rates reported from Kansas City, for example, and other high-rate urban areas.[3] The rates for the rest of Dalton (Section *B*) appear to be close to 4 to 5 per cent per year.

* * *

Numerous investigators have reported variation in the prevalence of histoplasmin sensitivity within the borders of single counties.[4-6] The present study also shows variations within counties and surprising variation withi: the limits of a small city. As far as the present writers are aware, this is the first time that higher reactor rates have been found in an urban population than in the local rural population. In view of the present results, it would appear that whatever the conditions are that define rural residence—low population density, intimate and frequent contact with soil, crops, and animals, and perhaps other factors—these conditions are not necessary for sensitization to histoplasmin.

The possibility was considered that residents of Section *A* might have acquired their histoplasmin sensitivity outside the city. The closest known high prevalence area of histoplasmosis which is readily accessible to Dalton residents is Tennessee. The responses to the high school questionnaire, however, showed no relationship between the frequency of histoplasmin reactors and time spent in Tennessee.

While temperature, humidity, and winds may play a role in the variation of histoplasmin sensitivity over large distances, they cannot account for the variations shown within the three miles across Dalton. The inescapable conclusion is that for some reason *H. capsulatum* grows, or grows

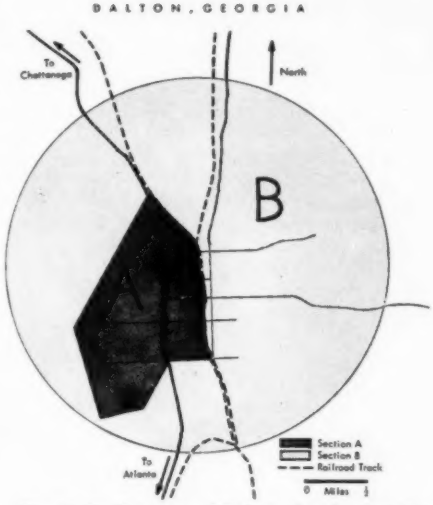

FIG. 2. Outline map of Dalton showing location of Sections *A* and *B*.

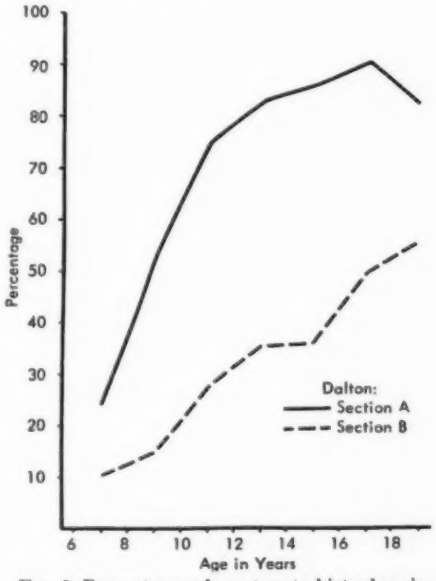

FIG. 3. Percentages of reactors to histoplasmin, by age, among schoolchildren living in Sections *A* and *B* of Dalton.

[3] MANOS, N. E.: Am. J. Hyg., 1953, *58*, 119.

[4] FURCOLOW, M. L.: Ann. New York Acad. Sc., 1958, *72*, 127.

[5] ZEIDBERG, L. D., *et al.*: Am. J. Pub. Health, 1951, *41*, 80.

[6] EDWARDS, L. B., *et al.*: Pediatrics, 1958, *21*, 389.

better, in one particular section of the city.[7] The laboratory growth requirements of the fungus are simple,[8, 9] but it may be that for survival in nature the organism has more selective requirements. Unfortunately, little is known of the soil chemistry of this area. However, the section in Dalton with the highest histoplasmin sensitivity rate is said to have more "fertile" soil than the rest of the city.[10] Responses to both questionnaires show that this is also the section where the most fertilizer is used. These are chiefly commercial inorganic mixtures and chicken manure.

Chicken manure and old chicken houses have been frequently implicated as the point source in epidemics of acute histoplasmosis, and many isolations of the organism have been made from chicken coops. It is not likely, however, that chicken litter is associated with the high reactor rate found in one section of Dalton. On the opposite side of town, where there are relatively few histoplasmin reactors, many people raise chickens in their yards. And in the surrounding counties, where the histoplasmin reactor rate is even lower, some ten million chickens, mostly broilers, are now being raised each year. (Ten million broilers produce thirty-five million pounds of manure.)

All soil cultures were reported negative by Dr. Ajello. Of 320 soil samples which were cultured, 91 were from chicken coops or from lawns fertilized with chicken manure.

None of the factors commonly associated with high histoplasmin sensitivity rates—rural residence, chickens, old houses, proximity to rural areas, positive soil cultures, and the like—seem to fit with the findings in the Dalton area. This raises the possibility that a great deal of the histoplasmin sensitivity in human populations may come from infective sources other than those in the soil. Moreover, the lower rates of histoplasmin sensitivity in the rest of Dalton and the even lower rates in surrounding rural areas suggest that this one section of the city may be the principal source of histoplasmin sensitivity throughout the two counties.

DAVID L. ARONSON
PHYLLIS Q. EDWARDS
Tuberculosis Program
Public Health Service
Department of Health, Education, and Welfare,
Washington 25, D. C.

September 4, 1958

[7] Since submission of the manuscript, a report of an epidemic in Wisconsin shows a similar variation in reactor rates in a small town. (WILCOX, K. R., *et al.*: Ann. Int. Med., 1958, *49*, 388.)
12-3-27

[8] PINE, L.: J. Bact., 1954, *68*, 671.

[9] SCHEFF, G. J.: Yale J. Biol. & Med., 1945, *18*, 41.

[10] DYER, H.: Personal communication.

REPORT ON THE USE OF ISONIAZID AND CYCLOSERINE IN 84 CASES OF PULMONARY TUBERCULOSIS

Standard drug combinations for the treatment of pulmonary tuberculosis in recent years have been: streptomycin-PAS, isoniazid-streptomycin, or isoniazid-PAS. There is an urgent need for additional drug regimens, because of: (*1*) the frequent appearance of drug resistance to streptomycin, as well as the occasional toxic side effects of this drug; (*2*) the rather frequent and severe gastric irritation produced by PAS; and (*3*) the experience with isoniazid which has shown that this drug alone is not sufficiently effective in adult pulmonary tuberculosis, especially when the disease is of long standing, unless an ancillary drug of proved effectiveness is added to it.

Other drugs and drug combinations have been tried in an effort to replace the combinations named above when, for one reason or another, they had lost their usefulness. Pyrazinamide-isoniazid was found useful but was associated with some hepatic toxicity.[1] A combination of oxytetracycline with either streptomycin or isoniazid has likewise been employed.[2] Other newer "miracle" drugs are still more or less in the experimental stage.

In this situation, a regimen of cycloserine and isoniazid has been found most useful,[3-9] and the results of treatment in 84 cases of pulmonary tuberculosis at the Rocky Glen Sanatorium have been sufficiently encouraging to warrant analysis and publication. To date, cycloserine and isoniazid have been used in more than 100 cases, the first 84 of which have been thoroughly analyzed.

Virtually all of the patients were chronic cases having an average duration of antituberculous treatment of four years (the longest period of sickness being seventeen years), and only 9 in the group had had prior antituberculous treatment for less than six months. Nine patients in the group had received, or are still receiving, therapeutic pneumoperitoneum—with or without phrenic crush; 5 had had compressive surgery, that is, thoracoplasty or extrapleural plombage, or both; and 3 patients had had resectional surgery (lobectomy). In addition, all had been under intensive antituberculous medication for prolonged periods of time with the three standard regimens of streptomycin-PAS, isoniazid-streptomycin, or isoniazid-PAS. These had been interchanged according to intolerance, toxic side effects, or the appearance of drug resistance. And pyrazinamide or oxytetracycline had been used in a few cases for limited periods of time when the three standard combinations could not be used. All but 9 patients had received prior combinations with isoniazid for periods ranging from six to twenty-four months.

The average dose in most cases has been two capsules a day, each with a combination of 250 mg. of cycloserine and 150 mg. of isoniazid, making a total daily dose of 500 mg. of cycloserine and 300 mg. of isoniazid. For only 3 patients was it necessary to interrupt this schedule temporarily, but it was possible to resume on the same regimen. For 3 other patients the dosage was temporarily decreased to one capsule of the combination a day. One patient received cycloserine without the isoniazid, since the isoniazid seemed to have toxic effects. One patient was started on one capsule, but the dose was soon increased to the regular treatment schedule of two capsules a day.

At the time when the study was compiled, this combination of drugs had been in constant use by 26 patients for a period of ten to fifteen months; by 31 patients, for a period of five to ten months; and by another 27 patients, for a period of less than five months. (By now there are more than 100 patients, some of whom have been under this treatment for almost two years.)

In 4 instances streptomycin was added to the treatment schedule at the rate of 1 gm. twice a week because of the presence of unusually toxic disease. The combination of cycloserine, isoniazid, and streptomycin was well tolerated by these 4 patients, none of whom showed unusual side reactions, and the therapy was most satisfactory and effective. In 2 cases in the series additional isoniazid was added to the treatment schedule because of body-weight requirements.

In 24 of the 84 cases the isoniazid-cycloserine therapy had to be discontinued because the patient was discharged from the sanatorium, left without medical sanction, or was transferred to

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[1] Muschenheim, C., *et al.*: Am. Rev. Tubere., 1954, *70*, 743.

[2] Bachman, H., and Freund, J.: Dis. Chest, 1957, *32*, 520.

[3] Harned, R. L., *et al.*: Antibiotics & Chemother., 1955, *5*, 204.

[4] Epstein, I. G., *et al.*: Antibiotic Med., 1955, *1*, 80.

[5] Epstein, I. G., *et al.*: Dis. Chest, 1956, *29*, 241.

[6] Epstein, I. G., *et al.*: Am. Rev. Tubere., 1957, *75*, 553.

[7] Robitzek, E. H., and Nenashev, P. K.: Antibiotics Annual 1956–1957, p. 431.

[8] Steenken, W., and Wolinski, E.: Am. Rev. Tubere., 1956, *73*, 539.

[9] U. S. Public Health Service: Am. Rev. Tubere., 1956, *74*, 196.

another institution. Two of these 24 patients, however, have returned and are again on the isoniazid-cycloserine therapy. Seven patients with far advanced disease died while under treatment. In 2 patients the therapy was discontinued because of other complications; one was an old man with heart disease who developed ascites and died, the other was a very old woman who showed an acute bronchogenic spread of her tuberculosis and remains in critical condition. Three patients refused to continue the drug for no apparently good reason. This leaves only 8 cases in which the isoniazid-cycloserine regimen had to be discontinued for strictly medical reasons. Three of these 8 patients complained of either dizziness, stomach trouble, or cardiac palpitation, or a combination of these complaints, and the remaining 5 simply stated that they could not tolerate it and felt worse while taking it.

Carefully evaluating the clinical progress in the series, improvement was definitely noted in 44 of the 84 patients. In 36 patients the disease remained static, the clinical symptoms changing but little under this treatment. Only 4 of the 84 patients have shown distinct decline, and 2 of these were patients with advanced disease who died within a period of two weeks to two months after admission to the sanatorium.

Tubercle bacilli disappeared from the sputum in 14 cases (verified by culture in 12 of the 14). This is considered a most significant result, inasmuch as the series consisted chiefly of patients with long-standing chronic disease who had received other drug combinations for a prolonged period of time before being put on isoniazid-cycloserine. In 6 cases the reversal of infectiousness occurred after one month of therapy; in 2, after two months; in one, after three months; in 2, after four months; in 2, after five months; and in one, after seven months.

In carefully evaluating the progress of the patients by roentgenography, it was found that in 40 of the 84 cases there had been a definite improvement, although this improvement was only slight or initial in 7 of the 40 cases. In another 40 cases of the series, there was no appreciable roentgenographic change, the pictures showing neither improvement nor spread. In only 4 cases of the series was the disease marked as being worse; and 2 of these patients died from advanced disease a rather short time after their admission to the sanatorium.

In further evaluating the clinical findings, it was found that the general well-being was distinctly improved in 44 of the 84 cases; the appetite improved in 35 cases; the weight increased in 29 cases (up to as much as 44 pounds), but 8 of the patients lost weight despite the treatment. Most of them were afebrile, but in one febrile patient a definite reduction in temperature was effected. Reduction in cough was frequent and was noted in 35 cases; reduction in expectoration was noted in an equal number of cases (36 patients). No extrapulmonary tuberculous complications were included in the series.

In checking for unpleasant side effects, it was found that 13 of the patients complained of minor discomforts: 3 felt slightly dizzy and had mild temporary stomach irritation; one patient complained of rather pronounced dyspnea, one of general malaise, one of itching; 4 complained of a feeling of tiredness and drowsiness. But these complaints usually disappeared after a very few weeks of treatment. One patient developed ascites for nonrelated reasons.

Urinalysis was done routinely and, except that the group contained 4 known diabetics and that there was one patient in whom pus cells were found in the urine, no kidney damage was noted in any case. The patient with pyuria also had skin itching, but both the itching and the pus cells disappeared after a short period of symptomatic treatment. Complete blood counts were done routinely and, except for one instance of decreased erythrocyte count, there were no significant changes. The erythrocyte sedimentation rate was carried out routinely, and a distinct decrease was found in 20 of the 84 cases; in only one case was there an increase in the sedimentation rate. The kidney function was further tested in all cases prior to the start of the treatment and was repeated whenever feasible. However, nitrogen retention did not interfere with treatment in any case.

Prior to initiating cycloserine therapy an attempt was usually made to determine the susceptibility of the patient's strain of tubercle bacilli to streptomycin and isoniazid. In 42 cases this was impossible since no culture could be obtained. In 13 cases, however, a distinct resistance to streptomycin had developed, and in another 19 cases some degree of resistance was present.

Complicating diseases did not seem to interfere with the treatment, and among the complications noted were severe arthritis, herpes zoster, alcoholism, appendicitis, glaucoma, asthma, cardiac conditions, cholelithiasis, drug addiction, neurasthenia, and peripheral vascular disease. None of these conditions interfered with the use of the isoniazid-cycloserine regimen, and the complicating conditions were not influenced by this therapy.

In summary: A series of 84 patients with pulmonary tuberculosis have been treated with isoniazid and cycloserine for periods as long as fifteen

months. Most of the patients had advanced chronic disease which had received the prior benefit of other drug combinations, including isoniazid in practically every case. Some of the patients had received compression or resectional surgery. In reviewing the results, a significant improvement was observed in a gratifying percentage of the patients. Reversal of infectiousness occurred in a satisfactory number of the patients; roentgenographic improvement was observed in a fair percentage; many patients showed improvement in general well-being and appetite; and there was a frequent reduction in cough and expectoration. Complicating side effects have been few and mild, despite the fact that the series included several diabetics and alcoholics. From this clinical experience, it is concluded that a regimen of 500 mg. of cycloserine and 300 mg. of isoniazid daily, in two equally divided doses, is a valuable regimen for use in patients who still have active disease despite treatment with one of the standard regimens.

HENRY BACHMAN
JULIUS FREUND
Rocky Glen Sanatorium,
McConnelsville, Ohio

April 30, 1958

CYCLOSERINE AND VIOMYCIN IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1]

A drug regimen consisting of cycloserine and viomycin has been tried in a group of tuberculous patients who were treatment failures with other forms of therapy. While it was not expected that such patients would be cured, it was hoped to reverse infectiousness and prevent relapse. It was hoped that this combination of drugs would mutually suppress the development of bacterial resistance. As will be shown, the regimen proved to be rather weak in reversing infectiousness, although the number of bacilli diminished in most cases, at least temporarily. It did well in holding the line in preventing relapse. While the resistance data will show a seeming delay in the development of resistance, particularly on the part of viomycin, the whole matter of the clinical significance of *in vitro* resistance to cycloserine and viomycin remained uncertain.

Cycloserine, 250 mg. twice daily every day by mouth (0.5 gm. daily), and viomycin, 1 gm. intramuscularly twice daily on two different days of the week (a total of 4 gm. a week), were given to 71 patients suffering from moderately or far advanced pulmonary tuberculosis. Treatment of the first patient was begun on June 2, 1957. All had tubercle bacilli in the sputum; all but 3 had demonstrable pulmonary cavities. All were treatment failures following the use of other drug regimens, surgical procedures when applicable, and bed rest. None had received cycloserine or viomycin previously. Chest roentgenograms, sputum smears and cultures, audiograms, complete blood counts, and liver and kidney function tests were done before treatment and at monthly intervals thereafter. Bacterial susceptibility to cycloserine and viomycin was determined for the strains of tubercle bacilli isolated. All of the patients were male. The distribution by age and race was about what might have been expected in a Veterans Administration hospital in the southeastern part of the United States.

Duration of treatment and losses to study: The duration of treatment of all of the patients is shown in detail in table 1. Forty patients received the regimen for at least four months. The 4 patients lost to the study died from causes which were essentially unrelated to chemotherapy. One patient died of cor pulmonale after a month of treatment. A second died after two months, following an abdominal operation unrelated to tuberculosis. A third died of a cerebral vascular accident after ten days of treatment. The fourth patient was moribund, suffering from extremely advanced pulmonary tuberculosis; although his roentgenograms showed no change, his course was steadily downhill and death occurred three weeks later. In only 2 cases was the regimen changed because of an increase of disease. It was changed in 9 others after six or more months because of an interest in trying these patients on a different regimen, but not because they had become intolerant to cycloserine or viomycin. One patient refused further treatment after receiving viomycin injections for a month; there was no evidence of toxicity in this instance.

Drug toxicity: The most disquieting form of drug toxicity was the occurrence of deafness in 3 patients. All 3 were known to have had partial hearing loss prior to treatment. This was found to have increased on audiometric examination after four months of treatment in 2 instances, and after five months in one. Sixteen other patients who were known to have hearing impairment at the beginning of treatment had no change in the findings of subsequent audiograms. Other forms of toxicity to viomycin occurred in 3 patients who developed allergic reactions consisting of skin rashes and, in one instance, of drug fever as well. Four patients had toxic reactions to cycloserine which consisted of one convulsion, one instance of drug fever, and 2 of nausea, all of which necessitated discontinuing the drug.

Therapeutic results: During treatment, 26 patients gained weight, 41 remained the same, and 4 lost weight. Sixty-two patients were afebrile. Eight who had fever before treatment subsequently became afebrile. In one, fever appeared after beginning treatment but disappeared upon continuation of therapy.

A review of pretreatment serial roentgenograms of patients who received the regimen for at least one month revealed the pulmonary disease to have been progressive in 15 and stationary in 52.

In table 2, which concerns the patients whose roentgenograms showed progressive disease before treatment, it may be seen that most patients showed considerable improvement by the third or fourth month. Relapse occurred in 3 patients. One with relapsing disease had steady roentgenographic deterioration for three months and later improved on the same regimen; the other 2 relapses occurred after satisfactory progress for five and eight months, respectively. Little effect of the therapy was seen on the cavitary component of the disease.

In table 3, those patients whose roentgenograms were stationary before treatment are set forth.

[1] Presented before the Medical Session, as part of *Section 1A*, at the annual meeting of the American Trudeau Society, Philadelphia, Pennsylvania, May 19, 1958.

TABLE 1

DURATION OF TREATMENT

Months	Cases
<1	3
1	5
2	16
3	7
4	5
5	7
6	8
7	6
8+	14

Relatively little evidence of roentgenographic improvement occurred and there was little change in cavities. Only one cavity disappeared. The one relapse consisted of a slight enlargement of a cavity with no increase of infiltration. As might have been expected, these cases showed little favorable change, due to the extreme chronicity of the disease and lack of reversible components.

The status of the bacillary content of the sputum is shown in table 4. While all of the patients had had tubercle bacilli in the sputum shortly before treatment was begun, the sputum examination made immediately before treatment was negative for tubercle bacilli in a number of instances. During the first several months of treatment, there was a somewhat gratifying diminution in the number of patients with sputum positive on microscopy. However, the high proportion of patients from whom bacilli could be cultured made it evident that this regimen was not a particularly powerful one in reversing infectiousness.

In table 5, the results of studies of bacterial susceptibility may be seen. Tests were performed in dilutions of 10, 20, and 50 γ of cycloserine per ml. and of 10 and 100 γ of viomycin per ml. on the majority of the strains of tubercle bacilli isolated from the patients. The table represents the drug susceptibilities of the patient-strains isolated at the designated periods of time. All drug-containing tubes had to show growth equal to that of the control to be considered positive in the appropriate dilution. The line marked "No Growth" indicates that, while growth occurred in the control tube, there was none in any of the tubes containing the drug. It may be seen that pretreatment growth in 10 γ of cycloserine per ml. was the rule, and was not unusual in 20 γ per ml. There was a slight tendency for resistance to cycloserine to increase with the passage of time. In the case of viomycin, growth in 10 γ per ml. was quite common prior to treatment and occurred in about half of the cases. There was little change in the proportion of patients whose strains of tubercle bacilli showed significant alteration in viomycin susceptibility. Thus, it is inferred that cycloserine has tended to prevent the emergence of viomycin resistance in this series of cases.

The trend of resistance was followed in individual patients on whom there were data for at least four months after the start of treatment. There were 24 such patients. In the case of cycloserine, the drug resistance of the patients' tubercle bacilli

TABLE 2

ROENTGENOGRAPHIC CHANGE

Disease Progressive Before Treatment

	Months							
	1	2	3	4	5	6	7	8
	Number of Patients							
Marked improvement	0	0	0	0	0	0	0	0
Moderate improvement	0	3	4	4	3	3	2	1
Slight improvement	4	5	4	1	1	1	1	1
No change	10	5	0	0	0	0	0	0
Worse	1	1	1	1	2	0	0	1
Cavity before treatment	15	14	9	6	6	4	3	3
Cavity same	12	6	2	1	1	1	1	1
Cavity smaller	3	7	6	4	4	3	2	2
Cavity gone	0	0	0	0	0	0	0	0
Cavity larger	0	1	1	1	1	0	0	0

TABLE 3

ROENTOGENOGRAPHIC CHANGE

Disease Stationary Before Treatment

	Months							
	1	2	3	4	5	6	7	8
	Number of Patients							
Marked improvement	0	0	0	0	0	0	0	0
Moderate improvement	0	1	1	1	1	1	1	1
Slight improvement	0	8	10	9	8	7	4	1
No change	52	39	22	19	16	11	7	4
Worse	0	0	1	1	1	0	0	0
Cavity before treatment	49	45	32	29	26	19	12	6
Cavity same	42	31	22	19	18	10	6	3
Cavity smaller	7	14	8	8	6	7	6	3
Cavity gone	0	0	1	1	1	1	0	0
Cavity larger	0	0	1	1	1	1	0	0

TABLE 4

PRESENCE OF TUBERCLE BACILLI IN SPUTUM

	Before Treatment	Months							
		1	2	3	4	5	6	7	8
	Number of Patients								
Microscopy negative	36	48	49	32	33	25	21	9	5
Microscopy positive	35	16	7	11	5	9	5	9	6
Culture negative	8	10	13	13	7	5	1	1	0
Culture positive	60	48	33	27	28	23	16	10	4

TABLE 5

DRUG SUSCEPTIBILITY STUDIES

γ per Ml.	Before Treatment	Months							
		1	2	3	4	5	6	7	8
	Number of Patients								
Cycloserine									
No growth	17	14	9	6	3	1	2	2	0
10	31	23	16	11	8	9	5	2	1
20	5	8	5	6	12	8	6	2	1
50	0	0	0	0	1	3	1	2	0
Viomycin									
No growth	25	19	11	9	7	10	3	4	1
10	28	26	19	14	17	11	11	4	1
100	0	0	0	0	0	0	0	0	0

increased in 18 cases, while in 6 there was no change. In the case of viomycin, only 9 patient-strains showed increasing resistance while 15 remained the same. Increased resistance to viomycin was within very narrow limits, since in no instance was any growth found beyond concentrations of 10 γ per ml.

The 4 patients whose disease relapsed were examined for possible correlation of the emergence of drug resistance and the occurrence of relapse. In one case it was found that the level of drug resistance of the patient's strain of tubercle bacilli had increased at the time of the relapse beyond that present before treatment. In a second case it had remained the same. In a third there were no data available. In the fourth case the disease was advancing, with the organisms completely susceptible in the early months of treatment while, in the later months, resistance to 50 γ of cycloserine per ml. and 10 γ of viomycin per ml. had occurred. By this time, however, the patient was showing steady improvement. Thus, any correlation between the emergence of resistance to cycloserine or viomycin and the occurrence of relapse could not be determined in this series of cases.

* * *

When an attempt is made to evaluate a chemotherapeutic regimen in pulmonary tuberculosis, the amount and type of disease present is of paramount importance. If the pulmonary involvement is slight and recent, it is likely to be readily reversible by bed rest alone—or perhaps with no treatment—and one is apt to arrive at a falsely enthusiastic assessment of the regimen. Conversely, when the disease is far advanced and very chronic, the regimen may be put to too severe a test. The patients in the present series had disease of a type that would provide an extremely severe test of drug efficacy. It was by taking this into consideration that the conclusion was reached that the combination of viomycin and cycloserine was of some value. The fact that most of the patients who had reversible disease showed roentgenographic clearing; the fact that the incidence of relapse was quite low, particularly for such an advanced group of cases; and, finally, the fact that the emergence of drug resistance (at least to viomycin) seemed

to be considerably delayed and actually prevented during the period of the study lent support to the idea that this regimen was a useful one. The only disquieting feature was the occurrence of deafness in 3 cases. Other drug toxicity was about the same as that which might have been expected with most drugs and sufficiently infrequent so as not to be objectionable.

In summary: Cycloserine and viomycin in a dosage of 0.5 gm. daily and 2 gm. twice weekly, respectively, given to 71 tuberculous patients who were treatment failures proved to be a useful regimen. This treatment may well be used as a second line of defense in patients for whom other chemotherapy is no longer effective. It is recommended, however, that audiograms or other careful hearing examinations be done in at least monthly intervals during treatment with this regimen.

WILLIAM S. SCHWARTZ
R. E. MOYER
Veterans Administration Hospital
Oteen, North Carolina

June 9, 1958

DETERMINATION OF GROWTH RATES OF ATYPICAL STRAINS AND OF OTHER MYCOBACTERIA AS A MEANS OF ESTIMATING OPTIMAL GROWTH PERIODS FOR COMPARATIVE BIOCHEMICAL STUDIES[1]

In a recent study of the lipid composition of Mycobacteria,[2, 3] saprophytic, atypical, avian, bovine, and human strains were to be examined. However, it was difficult to judge the time at which the different cultures had reached a comparable stage of growth. It is known that saprophytic, atypical, and avian strains grow more rapidly than human and bovine strains,[4-6] but no detailed data have been published concerning the atypical strains.

Therefore, in this paper, growth-rate constants and generation times of 8 atypical strains and of 9 other strains of Mycobacteria are compared, and the data obtained are used to establish the time at which Mycobacteria should be harvested for further biochemical studies.

Growth-rate constants and generation times: The technique used in this study is analagous to the method of Youmans.[7] For each of the cultures under study, five tubes of Tween®-albumin broth were inoculated with each of the following suspensions, using two drops of the suspension as the inoculum:

Suspension *a*—1:5 dilution of culture that has just reached the standard optical density of 0.05 (2 drops equal approximately 10^{-2} mg. of bacilli)

Suspension *b*—1:100 dilution of suspension (*a*); (2 drops equal 10^{-4} mg. of bacilli)

Suspension *c*—1:100 dilution of suspension (*b*); (2 drops equal 10^{-6} mg. of bacilli)

The weight of bacilli in each suspension was estimated from nephelometric measurements, using the data given by Smith.[8] The tubes were incubated at 37°C. and the optical density was measured daily. The time at which growth appeared in the tubes (optical density 0.005; this corresponds to the first appearance of macroscopic growth) was noted, and the growth rate constants and generation times were calculated as follows:

$$k = \frac{a - b}{t}$$

k = growth-rate constant
a = logarithm of the largest inoculum
b = logarithm of the smallest inoculum
t = difference between the time of appearance of growth in the tubes with the smallest inoculum and the time of appearance of growth in the tubes with the largest inoculum, expressed in days.

$$G = \frac{\log 2 \times 24}{k}$$

G = generation time (in hours)

It may be seen from table 1 that atypical strains have growth-rate constants which are situated between the growth rate constants of saprophytic strains and the growth-rate constants of human strains. Avian and atypical strains have similar growth-rate constants. Thus, these data permit the same conclusion as the neotetrazolium inhibition test,[9] namely, that it is impossible to differentiate atypical strains from avian strains by these methods.

Concerning the problem of harvesting Mycobacteria for further biochemical studies, Asselineau[12] mentions in his study of the lipids of tubercle bacilli that it would be advisable to harvest bacteria before they begin to autolyze in

[1] The present study is part of the cooperative study on atypical Mycobacteria sponsored by the Laboratory Subcommittee of the American Trudeau Society and has been aided by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; and from the Wisconsin Anti-Tuberculosis Association.

[2] Gastambide-Odier, M. M., *et al.*: Am. Rev. Tubere., 1957, *75*, 843.

[3] Smith, D. W., *et al.*: Ann. New York Acad. Se., 1957, *69*, 145.

[4] Robbins, E. S., *et al.*: J. Lab. & Clin. Med., 1955, *45*, 546.

[5] Fisher, M. W., and Kirchheimer, W. F.: Am. Rev. Tubere., 1952, *66*, 758.

[6] Miller, I. L., and Roessler, W. G.: Am. Rev. Tubere., 1956, *73*, 716.

[7] Youmans, G. P., and Youmans, A. S.: J. Bact., 1949, *58*, 247.

[8] Smith, D. G.: Proc. Soc. Exper. Biol. & Med., 1947, *64*, 36.

[9] Gastambide-Odier, M. M., and Smith, D. W.: Am. Rev. Tubere., 1958, 77, 662.

[10] Timpe, A., and Runyon, E. H.: J. Lab. & Clin. Med., 1954, *44*, 202.

[11] Buhler, V., and Pollak, A.: Am. J. Clin. Path., 1953, *23*, 363.

[12] Asselineau, J.: Prog. Explor. Tubere., 1952, *5*, 1.

TABLE 1

GROWTH-RATE CONSTANTS AND GENERATION TIMES OF ATYPICAL STRAINS AND OF OTHER MYCOBACTERIA

Strain	Growth-Rate Constant	Generation Time
		hours
M. phlei	4/1 = 4	1.8
M. smegmatis	4/1 = 4	1.8
M. fortuitum Cruz	4/1.5 = 2.7	2.7
Atypical strains*		
Nonphotochromogens		
Strain 17	4/3 = 1.3	5.6
Strains 2 and 7	4/3.5 = 1.1	6.6
Strain 3	4/4 = 1.0	7.2
Photochromogens		
Strains 1† and 4	4/4 = 1.0	7.2
Scotochromogens		
Strains 5 and 6	4/4 = 1.0	7.2
M. avium	4/4 = 1.0	7.2
Bovine strain (Rockefeller Institute #728)	4/5 = 0.8	9.0
BCG (Phipps)	4/5.5 = 0.7	10.3
H37Rv	4/5.5 = 0.7	10.3
H37Ra	4/6 = 0.66	10.9
Human strain (recent isolate)	4/8 = 0.5	14.4

* Strains and classification of the Laboratory Subcommittee of the American Trudeau Society. The classification is discussed by Timpe and Runyon.[10]

† Case 1—Buhler and Pollak.[11]

the period of their logarithmic phase of growth. To determine the growth curve for each culture directly on the synthetic medium of Wong-Weinzirl[13] would require very large amounts of medium and numerous culture containers. In Tween-albumin liquid medium, the growth curves of Mycobacteria are characterized by a long arithmetic linear growth-phase following the logarithmic phase of growth,[4, 5] and the beginning of autolysis is difficult to determine. Therefore it was concluded that it might be easier to establish harvesting periods by comparing growth-rate constants instead of growth curves.

If a human strain, cultivated in Dubos-Tween albumin medium, is harvested at a pre-established time before the period of autolysis (by experience it appears that at seven days a human strain is multiplying actively), the growth-rate constant data permit calculation as to when Dubos-Tween albumin cultures of other strains should be harvested. The following are examples:

[13] WONG, S., AND WEINZIRL, J.: Am. Rev. Tuberc., 1936, *33*, 577.

Strains	Growth-Rate Constant (Tween Albumin Medium)	Growth Period (Tween Albumin Medium)
		days
Human strain	0.7	7 (pre-established)
Atypical strain *M. phlei*	4	1.5 (calculated)

Although it is not possible to establish a direct relationship between the diffuse type of growth in Tween-albumin medium and the surface growth on Wong-Weinzirl medium, it seems that the growth-rate constants measured in Tween albumin medium can be used to determine approximate harvesting periods of cultures on Wong-Weinzirl medium. It has been observed in the laboratory that a mass culture of H37Rv on Wong-Weinzirl medium is still in its logarithmic phase of growth at about twenty-eight days. Considering this the optimal time for this culture, it is possible to calculate the harvest times for several other strains as follows:

Strain	Growth-Rate Constant	Growth Period for Mass Culture
		days
H37Rv	0.7	28 (pre-established)
M. avium	1.0	20 (calculated)
M. smegmatis	4.0	5 (calculated)

Data obtained on Wong-Weinzirl medium with *M. phlei* and *M. smegmatis* confirm these estimates. Eight bottles of each strain were harvested four, eight, and sixteen days after inoculation, and the dry weight of the cells was determined.

	4 days	8 days	16 days
M. phlei	16.62 gm.	20.02 gm.	20.22 gm.
M. smegmatis	14.76 gm.	24.47 gm.	21.83 gm.

It may be seen that the bacterial weight increases between four and eight days and is stationary or decreases after eight days. These data would have induced the writers to harvest the bacteria between four and eight days. From the growth rate it would have been calculated that five days would be the right growth time for these cultures. Therefore it appears that the growth-rate data can be used to calculate harvesting times for cultures grown on Wong-Weinzirl medium, but further experiments will be necessary to establish this fact with more certainty.

Gutiérrez-Vázquez[14] presented data on the direct measurement of growth-rate constants and generation times of H37Rv on several different types of media, including three liquid synthetic media.

In summary: The atypical and avian strains have growth-rate constants that are intermediate between the growth-rate constants of saprophytic strains and the growth-rate constants of human strains. A simple method is devised to estimate the time at which Mycobacteria should be harvested for further biochemical studies.

MIREILLE GASTAMBIDE ODIER[15]
D. W. SMITH,
Department of Medical Microbiology, University of Wisconsin, Madison, Wisconsin

March 14, 1958

[14] GUTIERREZ-VAZQUEZ, J. M.: Am. Rev. Tuberc., 1956, 74, 50.

[15] Present address: Institute de Biologie Physico-chimique, 13 Rue Pierre Curie, Paris V^e^, France.

ACQUIRED RESISTANCE AND ISONIAZID MEDICATION

In the recent years, several papers have been published concerning the influence of isoniazid therapy on acquired resistance. The problem has been discussed in relation to two questions: (*1*) Is bacterial multiplication important for the development of immunity? (*2*) Does isoniazid prophylaxis interfere with this development?

To test the existence of immunity in heavily infected isoniazid-treated mice, various writers[1-4] superinfected the animals at intervals after therapy was terminated. McCune and Tompsett[5] have shown that, in mice, a remarkable number of organisms remain viable despite prolonged high doses of isoniazid given simultaneously with infection (10^3 to 10^4 bacterial units per ml. of homogenized spleen). In similar experiments but using a different strain of mice (Swiss-Rockefeller, kindly supplied by Dr. R. J. Dubos) and a different culture for challenge (Washington I) equal results were obtained. On the other hand, Bloch and Segal[2] and Dubos and Pierce[6] demonstrated a certain degree of protection against challenge with virulent organisms three days after vaccination with high doses of BCG or fourteen days after vaccination with only 6×10^3 BCG units. Therefore, there is the possibility that the number of bacteria surviving isoniazid treatment may be sufficient to elicit immunity in this short time provided that the germs multiply normally. Thus, in evaluating the experiments mentioned above there is no certainty that animals challenged even shortly after the end of therapy developed immunity *during* isoniazid therapy or *after*.

To differentiate between these possibilities challenge has to be carried out immediately after termination of therapy, as was done by Bloch and Segal in one of their experiments on heavily infected mice. Under those conditions, immunity was demonstrable, too; but in experiments with a small infecting dose, isoniazid-treated mice did not show signs of immunity.[4, 7, 8]

Similar statements can be made about experiments on guinea pigs. Acquired resistance also develops quickly after massive infection. Grüner and Hamburger[9] observed a Koch phenomenon six days after virulent infection; Debré and Bonnet,[10] after fifteen days. In guinea pigs infected with minimal doses, immunity requires up to four weeks to develop, as shown by Lange and Brunzema.[11]

Usually isoniazid therapy does not eradicate all bacteria after massive infection,[12-14] and the surviving germs may build up an immunity within the four weeks following the termination of treatment. Therefore, the moment at which immunity has arisen cannot be determined if superinfection has been made two to four weeks or later after therapy was terminated, as was done in the experiments of Ferebee and Palmer,[15] of Palmer and associates,[16] and of Sher and associates.[14] The same can be said about the majority of the superinfected animals in the studies of Peizer and associates.[17] But in the experiments of Bretey and Canetti[18] and of Jankov,[19] the guinea pigs inoculated with a high dose of BCG and simultaneously treated with isoniazid were superinfected at the end of therapy. Their resistance corresponded well to that of animals which were vaccinated but not treated.

The following study was made to elucidate the development of immunity in guinea pigs during

[1] Hirsch, J.: Naturwissenschaften, 1953, *40*, 490.

[2] Bloch, H., and Segal, W.: Am. Rev. Tuberc., 1955, *71*, 228.

[3] Dubos, R. J., *et al.*: Exper. Med., 1953, *97*, 207.

[4] Dubos, R. J., and Schaefer, W. B.: Am. Rev. Tuberc., 1956, *74*, 541.

[5] McCune, R. M., and Tompsett, R.: J. Exper. Med., 1956, *104*, 737.

[6] Dubos, R. J., and Pierce, C. H.: Am. Rev. Tuberc., 1956, *74*, 699.

[7] Robson, J. M., and Didcock, K. A.: Brit. J. Pharmacol., 1956, *11*, 190.

[8] Bloch, H.: in *Experimental Tuberculosis*, edited by G. E. W. Wolstenholme and M. P. Cameron, J. and A. Churchill, London, 1955, p. 245.

[9] Grüner, O., and Hamburger, F.: Beitr. Klin. Tuberk., 1910, *17*, 1.

[10] Debré, R., and Bonnet, A.: Compte rend. Soc. de biol. Paris, 1922, *87*, 449.

[11] Lange, B., and Brunzema, B.: Ztschr. Hyg., 1930, *111*, 354.

[12] Bartmann, K., *et al.*: Am. Rev. Tuberc., 1956, *74*, 475.

[13] Sher, B. C., and Czaja, Z. G.: Tr. Illinois Acad. Sc., 1956, *48*, 92.

[14] Sher, B. C., *et al.*: Am. Rev. Tuberc., 1957, *75*, 295.

[15] Ferebee, S. H., and Palmer, C. E.: Am. Rev. Tuberc., 1956, *73*, 1.

[16] Palmer, C. E., *et al.*: Am. Rev. Tuberc., 1956, *74*, 917.

[17] Peizer, L. R., *et al.*: Am. Rev. Tuberc., 1957, *76*, 732.

[18] Bretey, J., and Canetti, G.: Ann. Inst. Pasteur, 1957, *92*, 441.

[19] Jankov, M.: Rev. tuberc. Paris, 1957, *21*, 303.

TABLE 1

SURVEY OF GROUPS EMPLOYED

Group	Number of Animals	Infecting Dose (bacterial units)		Remarks
		P.I.*	S.I.†	
I*a*	5	23	—	Controls designated to check the virulence of the first infection in the right thigh
I*b*	5	6 × 23 each	—	Controls to demonstrate the presence of bacteria in the inoculating dose of the minimal primary infection
II	10	23	343	Infected as group I*a*, treated subcutaneously with isoniazid (10 mg. per kg., twice weekly)
III	10	4.58×10^5	343	Treated as group II
IV	10	4.58×10^5	343	Treated subcutaneously with isoniazid (10 mg. per kg. six times weekly)
V*a*	10	—	343	Vaccinated with BCG and superinfected
V*b*	5	—	—	Vaccinated with BCG as group V*a*; not superinfected
VI	10	—	343	Controls designated to check the virulence of the superinfection, inoculated subcutaneously into the neck
VII*a*	5	17	—	Animals not treated; infected in similar manner to groups I*a* and II, at the end of therapy in the treated group
VII*b*	5	17	343	Handled like VII*a*; in addition, infected with the superinfecting dose

* P.I. = primary infection.
† S.I. = superinfection.

isoniazid therapy after massive and minimal infections with a virulent strain of *M. tuberculosis*.

The animals were divided into groups as shown in table 1. Each animal of group I*b* received six injections of the small first infecting dose of tubercle bacilli distributed over the back to make sure that the isoniazid-treated minimally infected animals of group II were really infected and that prevention of manifest disease in group II was due to therapy. As mentioned before, the investigation had to take into account that in some of the animals tuberculosis could spread from the treated primary infection concomitant with the superinfection and would give a protection against the latter. Therefore, the groups VII*a* and *b* were formed. Group VII*b* should demonstrate to what extent the tuberculosis caused by the superinfecting dose (*cf.* group VI) could be influenced by a minimal infection (*cf.* group VII*a*) simultaneously employed. For BCG vaccination, the strain Copenhagen was used which had been grown on potato-glycerin bouillon. The animals received 1.17×10^7 units subcutaneously four months before challenge, and again 3.32×10^7 units intraperitoneally two months before challenge. The wet weight of each dose was about 1 mg. Group V*b* was introduced to demonstrate the changes induced by the so-called "Becegitis" (Calmette) alone. All virulent infections were carried out with the human strain Washington I.

To obtain a suspension as homogenous as possible the following procedure was adopted: Several clumps from a three-week-old culture on Hohn egg slant were shaken for a short time by hand in Tween®-albumin medium. After several hours the supernatant was pipetted off, kept in the refrigerator overnight, and then centrifugated at $140 \times g$. The supernatant was pipetted off and used for inoculation. The homogeneity was good, as controlled by staining. The suspension consisted of 86 per cent single cells in the first infection and of 79.9 per cent in the second infection. The bacterial content was determined in a bacterial counting chamber by phase contrast microscopy. The desired concentrations of bacteria were obtained by dilution in 0.2 per cent bovine albumin, fraction V (Armour). The percentage of viable units was determined by culture on egg slants (10 slants for each dilution tested). The isoniazid susceptibility of the concentrated bacterial suspension was tested on egg slants. The growth was inhibited by 0.03 γ of isoniazid per ml. Therapy was started two hours before infection and was continued for four months. The dosage of isoniazid was corrected weekly according to weight change. The day following termination of therapy the animals were superinfected (resp. infected in groups VII*a* and *b*). The infection was made by the respiratory route in groups VII*a* and *b*. Seven weeks later all surviving animals were killed and examined at autopsy. The extent of macroscopically visible disease in spleen, liver, and lungs was classified, using an index from 0 to 4. The sum of the indices gave the total index of each animal. The sites of infection and the regional lymph nodes were carefully examined. During the experiment the animals were examined once or twice weekly, and the findings were recorded. The tuberculin sensitivity was checked at certain intervals.

The test strain was found to be highly virulent, as in former experiments. All controls infected

TABLE 2

AUTOPSY FINDINGS

Group	Number of Animals	Index of Organs		Extent of Primary Tuberculosis		
		Mean	Possibly Influenced by Primary Infection	Number of Lymph Nodes*		Number of Abscesses*
				Tuberculous	Nontuberculous	
Group II						
without lesions	4	3.00	0	2	14	0
with lesions	6	5.83	5	12	14	1
Group III						
without lesions	4	1.25	0	1	15	0
with lesions	5	0.80	4	19	8	2
Group IV						
without lesions	6	4.00	0	0	25	0
with lesions	3	0.50	0	4	12	0
Group V*a*	9	1.22	—	—	—	—
V*b*	4	1.00	—	—	—	—
Group VI	10	5.60	—	—	—	—
Group VII*a*	5	2.40	—	31	2	5
VII*b*	5	2.40	—	27	9	4

* Per group.

with the minimal dosage to test the virulence of the infective strain died with signs of far advanced disease on the one hundred and second, one hundred and twenty-ninth, one hundred and thirtieth, one hundred and fifty-third, and one hundred and seventy-second day, respectively, after infection. The animals of group I*b*, each inoculated six times in the back with the minimal infecting dose, were killed after three months. Each of the thirty sites of inoculation showed abscess formation. Therefore, it may safely be assumed that all animals of group II, inoculated with the same minimal dose and *treated*, were really infected.

A detailed description of the findings of the present writers at the site of inoculation and on tuberculin tests may be found in a previous report.[20] It may be sufficient to mention that the various groups reacted similarly to the previous experimental groups with regard to formation of abscesses, transient enlargement of lymph nodes, and tuberculin tests. The observations made during the period of superinfection will not be discussed here because they are largely in agreement with the results of the autopsy examinations.

The total indices of the lesions of organs (mean values) are shown in table 2. All treated groups were divided in two subgroups. The first one comprised the animals with macroscopically visible disease at the site of primary infection; the second, those with minimal or no signs of tuberculosis in this region. The fourth column gives the number of animals in which the total index is possibly caused not only by the superinfection but by the primary infection too. This possibility was accepted if the primary infection had progressed to the iliacal lymph nodes. The extent of the primary tuberculosis is indicated in the last two columns showing the number of tuberculous lymph nodes and abscesses at the site of primary infection.

The control group for the superinfection (group VI) had an index of 5.60; group VII*a*, of 2.40. The latter group of animals received a minimal infection comparable to the primary infection in group II at the time when therapy was terminated.

Group VII*b* had an identical index of 2.40 despite the fact that the animals in this group had been infected simultaneously with the dose of superinfection used in group VI and the minimal infecting dose used in group VII*a*. This seems to indicate that these two infections exerted an inhibitory influence upon each other. Evaluating the degree of protection against superinfection of guinea pigs pretreated with isoniazid, it must be realized that multiplication of a few surviving tubercle bacilli starting with normal speed at the termination of pretreatment will be sufficient to slow down the spread of the subsequent challenge infection. Therefore, each pretreated group was divided into two subgroups: one with marked disease at the site of primary inoculation (henceforth referred to as subgroup "with lesions"), and the other with minimal or no signs of primary tuberculosis (referred to as subgroup "without lesions") so that the influence of the primary infection

[20] BARTMANN, K., *et al.*: Beitr. Klin. Tuberk., 1958, *118*, 87.

on the superinfection could be considered immaterial. In group III, animals infected with massive doses and treated twice weekly with isoniazid, the subgroup "without lesions" showed an index significantly below that of the controls of group VI and even lower than that of group VII*b*, which had received two primary infections.

In the subgroup "with lesions" the primary tuberculosis showed less spread than group VII*b*, notwithstanding the fact that the index of this subgroup reached only 0.80, whereas that of group VII*b* was 2.40. Therefore, it may be assumed that the immunity in group III developed during therapy and not thereafter. In group IV, animals heavily infected and treated six times weekly, the subgroup "without lesions" had an index of 4.00, which is lower than that of the controls for superinfection (group VI), 5.60. Here too, the primary infection in the subgroup "with lesions" was not as far advanced as in group VII*b*. Nevertheless, the index of this subgroup was lower than that of group VII*b* (0.50 versus 2.40). Obviously, immunity developed in group IV during therapy. The group index of group IV (isoniazid six times weekly) at 2.44 is higher than that of group III (isoniazid twice weekly), at 1.00.

In the former studies of the present writers it has been shown that continuous treatment of manifest disease is somewhat more effective than an intermittent regimen. In the present experiment, the same conclusions may be drawn with regard to the development of abscesses, the reaction of regional lymph nodes, and the tuberculin test. The findings seem to indicate that a more effective chemotherapy results in a lesser degree of immunity. These observations are in agreement with those of Ferebee and Palmer[15] and Palmer, Ferebee, and Hopwood.[16] In many chemotherapeutic experiments on animals, the results are measured by the velocity of the spread of tuberculosis *after* termination of therapy (in terms of survival time or extent of pathologic changes). In this type of experiment, therapy with smaller doses in certain circumstances can give better results than with larger ones—at least in guinea pigs—because smaller doses depress immunity less than larger doses. The results obtained by the present writers confirm these observations.

In a survival experiment described in a previous report[21] and mentioned in a Letter to the Editor of the REVIEW,[12] the animals were examined at autopsy thirteen weeks after termination of therapy (10 mg. of isoniazid per kg. twice or six times daily for three months, or the same dose plus 20 mg. of streptomycin per kg. plus 0.2 gm. of PAS per kg., commencing at the time of infection). The index of lesions in the groups that received intermittent treatment was half that in the continuously treated groups. In another experiment, advanced tuberculosis was treated in the same way, but the animals were killed at the end of therapy. Macroscopic, histologic, and cultural findings showed that continuous therapy was superior. Corresponding observations were published by Zebrowski and co-workers.[22]

Group II is especially interesting, for it duplicates conditions present in natural infection with respect to the infecting dose. At first glance it may seem surprising that it is just the subgroup "with lesions" which is completely unprotected against superinfection (index 5.83 versus 5.60 in group VI or versus 2.40 in the control group VII*b*), whereas the subgroup "without lesions" is protected almost as well as by a virulent minimal infection (index 3.00 versus 2.40 in group VII*a* and *b*). This may be explained by the results of one of the former experiments in which the influence of isoniazid treatment in minimal infection was examined.[23] It was found at that time that, after termination of therapy, those guinea pigs which showed a transient enlargement of their regional lymph nodes and/or a tuberculin reaction relatively early during treatment remained without overt disease. Because these phenomena are often linked with immunity, it was assumed that the success of prophylactic treatment was partly caused by the development of immunity. The experiment described herein confirms this view.

The subgroup "without lesions" contained all of the animals of group II which became tuberculin positive during therapy and 2 of the 3 animals which showed transient enlargement of lymph nodes. This subgroup also contained the majority of those animals which appeared to be protected against superinfection. Under the conditions of the experiment, the small antigenic stimulus of a minimal infection prophylactically treated with isoniazid was sufficient to develop immunity in some animals during therapy. These animals remained free of overt disease.

Obviously the individual capacity to build up immunity demonstrates whether intermittent isoniazid therapy is successful or not. Individual differences in the ability to develop immunity are clearly shown in Jespersen's experiments with

[21] BARTMANN, K., *et al.*: Beitr. Klin. Tuberk., 1957, *116*, 471.

[22] ZEBROWSKI, T., *et al.*: Beitr. Klin. Tuberk., 1956, *115*, 421.

[23] BARTMANN, K., *et al.*: Am. Rev. Tuberc., 1958, 77, 999.

guinea pigs, using graded doses of BCG.[24] By raising the BCG dose from 10^{-5} to 10^{-1} mg., the number of protected animals became greater but not their degree of resistance measured in terms of survival time. It is not believed that immunity alone is responsible for prevention of tuberculosis in the experimental study. Immunity could only retard the spread of the small superinfecting dose (343 bacterial units). Therefore, an infection with 20 to 30 bacilli of the present challenge strain would hardly be prevented by immunity. The individual ability to develop immunity could explain why those animals remained unprotected against superinfection in which primary infection became manifest after therapy and could be expected to have induced immunity.

Minimally infected animals are divided by isoniazid administration into two groups with good and with poor ability to become immunized. If immunity plus isoniazid makes prophylaxis successful, animals with a low degree of immunity cannot cope with minimal infection and fall therefore into the subgroup of animals "with lesions." The poor ability to become immunized is the reason, too, why this subgroup is not protected against superinfection despite manifest primary infection. It remains an open question why isoniazid therapy of minimally infected guinea pigs left immunity in some of the animals whereas weakly infected and isoniazid-treated mice did not show immunity in the experiments mentioned above. These differences may be due to species differences or to differences in therapy (the mice were treated continuously; the minimally infected guinea pigs received intermittent treatment). As in the studies cited above, the findings in the present study do not permit the conclusion that an infection corresponding to natural conditions with respect to the infecting dose leads to development of sufficient immunity despite isoniazid medication.

The better results in the subgroup "without lesions" might be explained on a different basis. Possibly the prophylactically treated animals which did not develop tuberculosis would respond less to isoniazid than others, so that the bacilli could multiply to a certain extent and the immunity elicited thereby would be sufficient to prevent manifestation of disease after termination of therapy. The observations of the present writers do not favor this hypothesis. In preliminary experiments, guinea pigs minimally infected and prophylactically treated which showed enlargement of lymph nodes and tuberculin reactions did not show evidence at autopsy of having had an initial generalization as they did after immunization with BCG. But this question must be answered by histologic examination and quantitative culture.

Under the present experimental conditions, protection against superinfection caused by the treated primary infections was less than that given by BCG vaccination. The animals of group V*a* (BCG plus superinfection) showed an index of 1.22; those of group V*b* (BCG only), 1.00. That means superinfection accounts for an index of 0.22 only.

In summary: An investigation has been carried out to discover whether development of immunity is possible during prophylactic isoniazid treatment in guinea pigs infected with tubercle bacilli and to what extent it depends upon the infecting dose and the type of treatment. Immunity has been measured in terms of protection against a small challenge dose with a highly virulent strain. It was found that after treatment of a massive infection with isoniazid (10 mg. per kg. twice weekly every third or fourth day, subcutaneously) immunity was demonstrable which must have been developed during therapy. It was somewhat less effective than that following BCG vaccination. If the same virulent primary infection was treated with isoniazid (10 mg. per kg. six times weekly), weaker immunity developed corresponding in degree to that produced by a progressive untreated minimal infection. In a minimal infection prophylactically treated with isoniazid (10 mg. per kg. twice weekly), considerable interference with the immunizing process was observed, i.e., only some of the animals became immune and were less protected than would be the case in a progressive minimal infection. Generally, the immune animals of the minimally infected group did not show signs of tuberculosis at the site of primary infection after therapy. This seems to indicate that under the conditions of the experiment, interplay between chemotherapy and the development of immunity prevented the appearance of "open" tuberculosis.

K. Bartmann
J. Villnow
Ch. Schwarz
Landes-Tuberkulose-Krenkenhaus
Heckeshorn, Berlin-Wannsee
Germany

June 6, 1958

[24] Jespersen, A.: Acta path. et microbiol. scandinav., 1956, *38*, 203.

PYRAZINAMIDE IN LOW DOSAGE IN COMBINATION WITH ISONIAZID OR PARA-AMINOSALICYLIC ACID IN THE TREATMENT OF PULMONARY TUBERCULOSIS

Since the earliest availability of pyrazinamide in 1952, the writer has been studying this drug in the treatment of pulmonary tuberculosis, first alone,[1] and later in low dosage in combination with isoniazid.[2] The hepatotoxicity of pyrazinamide was dramatically recorded by McDermott and associates who had one death from progressive hepatitis among six patients who had unequivocal hepatitis in the group of fifty-five patients studied who received full-dosage (50 mg. per kg. of body weight) pyrazinamide in combination with isoniazid (5 mg. per kg. of body weight).[3] The therapeutic effect from the combination was so good, however, that more work was done by various clinicians, using this large dosage of pyrazinamide. The jaundice rate in these studies usually approached 10 per cent. The present writer believed the large dosage of pyrazinamide was too hepatotoxic, and he has since used only low dosage (1.5 gm. daily).

With the low dosage, the over-all incidence of jaundice has been 1 per cent and the therapeutic result only slightly less than when the large dosage was used. Even with the orthodox forms of therapy, there was a considerable incidence of drug-resistant strains among the patients who were still discharging tubercle bacilli after eight months of treatment. It appeared, however, that pyrazinamide in low dosage, combined with isoniazid, was a very adequate regimen for short periods (not to exceed eight months). Although less hepatotoxic than when used in high dosage, nevertheless, pyrazinamide was toxic even in low dosage. Consequently, when the less toxic drugs were available, it seemed best that pyrazinamide should not be used except in treating patients whose organisms were resistant to streptomycin and/or isoniazid.

It was the purpose of the present investigation to explore the lower dosage of pyrazinamide in association not only with isoniazid but also with para-aminosalicylic acid (PAS), with the hope that the latter regimen might be effective in patients whose organisms were isoniazid resistant. However, the studies of these regimens include only previously untreated patients in the present report. No patient was accepted for the study who had previously had hepatitis, cirrhosis, or who was a confirmed alcoholic. Liver function screening was done on each patient before acceptance for the study, and any with abnormal tests were unacceptable. After the pretreatment screening, liver function tests were done every two weeks until the study was completed or until abnormal elevations precluded continuance of the study in any individual. The liver function tests included: quantitative serum bilirubin, thymol turbidity, and cephalin flocculation. If any of these values became elevated, a bromsulfalein test was often done and, if there was abnormal retention, the patient was dropped from the study and pyrazinamide was discontinued. The patients were chosen by randomization based upon the last digit of their hospital registry number. The three regimens for study were:

Regimen I. Pyrazinamide, 1.5 gm., plus isoniazid, 300 mg. daily

Regimen II. Isoniazid, 300 mg., plus 12 gm. of PAS daily

Regimen III. Pyrazinamide, 1.5 gm., plus 12 mg. of PAS daily

Patients whose registry number ended in 1, 2, or 3 were given *Regimen I*. Those whose registry number ended in 4, 5, or 6 were given *Regimen II*; and those whose registry number ended in 7, 8, 9, or 0 were given *Regimen III*. In *Regimen I* there were 50 patients; in *Regimen II* there were 51 patients; and in *Regimen III*, 63 patients.

A total of 164 patients, all previously untreated, were treated with the three regimens as follows: 50 patients were treated with *Regimen I* (1.5 gm. of pyrazinamide and 300 gm. of isoniazid); 51 patients, with *Regimen II* (300 gm. of isoniazid and 12 gm. of PAS); and 63 patients, with *Regimen III* (1.5 gm. of pyrazinamide and 12 gm. of PAS).

In the total of 164 patients for the three groups there were 47 with far advanced disease, 85 with moderately advanced disease, and 32 with minimal disease. In *Regimen I* there were 15 with far advanced disease, 22 with moderately advanced disease, and 13 with minimal disease. In *Regimen II* there were 16 with far advanced disease, 25 with moderately advanced disease, and 10 with minimal disease. In *Regimen III* there were 16 with far advanced disease, 38 with moderately advanced disease, and 9 with minimal disease.

In *Regimen I* (a total of 50 cases), 33 patients

[1] Allison, S. T.: Tr. Twelfth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1953, p. 396.

[2] Allison, S. T.: Am. Rev. Tuberc., 1956, *74*, 400.

[3] McDermott, W., *et al.*: Am. Rev. Tuberc., 1954, *69*, 319.

TABLE 1

THE RESULTS OF TREATMENT IN THOSE PATIENTS COMPLETING EIGHT MONTHS OF CHEMOTHERAPY, TOXICITY NECESSITATING WITHDRAWAL OF DRUGS, AND DRUG RESISTANCE

	Regimen I (1.5 Gm. of Pyrazinamide plus 300 Mg. of Isoniazid)	Regimen II (300 Mg. of Isoniazid plus 12 Gm. of PAS)	Regimen III (1.5 Gm. of Pyrazinamide plus 12 Gm. of PAS)
Number of cases	50	51	63
Reversal of infectiousness (culture)	23 (46%)	28 (54.9%)	30 (47.6%)
Substantial roentgenographic improvement (moderate or marked)	25 (50%)	23 (45%)	22 (35%)
Cavity closure (on drugs alone)	(60%)	(60%)	(70%)
Worsening	2 (4%)	2 (4%)	3 (4.7%)
Died	0	0	1
Drug resistance (to isoniazid)	8 (100% of those with culture still positive)	1 (33⅓% of those with culture still positive)	Not done
Toxicity	4 (8%)	5 (9.8%)	3 (4.7%)
Jaundice	1 (2%)	0	0

completed eight months of therapy. Seventeen patients on this regimen were dropped from the study for the following reasons: 4 because of abnormal liver function tests; 2 because of worsening, necessitating a change of drug regimens; 7 because of discharge from the hospital against medical advice; 4 because of discharge from the hospital by maximal hospital benefit, some drugs (never pyrazinamide) to be continued at home.

In *Regimen II* (a total of 51 cases), 32 patients completed eight months of therapy. Nineteen patients on this regimen were dropped from the study for the following reasons: 5 because of drug reactions, mostly due to PAS; 2 because an additional drug (streptomycin) was added because of lack of improvement; 11 because of discharge against medical advice; one because of a "maximal hospital benefit" type of discharge.

In *Regimen III* (a total of 63 cases), 34 patients completed eight months of therapy, and 29 patients on this regimen were dropped from the study as follows: 12 because of PAS reactions or gastrointestinal intolerance; 3 because of roentgenographic spread of disease; 3 because of abnormal liver function tests; 8 because of discharge against medical advice; one because of transfer to another hospital; one died (acute myocardial infarction); one because of maximal benefit discharge.

Table 1 is a summary table showing the results of treatment in the patients who completed eight months of chemotherapy, the toxicity which necessitated withdrawal of drugs, and drug resistance.

* * *

It may be seen that on *Regimens I* and *III*, in which pyrazinamide was one of the companion drugs, 7 patients were dropped from the study and the drugs were discontinued because of increasingly abnormal liver function tests. There were 50 patients on *Regimen I* and 63 patients on *Regimen III*, or a total of 113 patients who received pyrazinamide as a companion drug. Since 7 patients had to be dropped from this number, the incidence of hepatic toxicity was 6.2 per cent. In this group of 113 patients receiving pyrazinamide, only one developed jaundice, so that the over-all incidence of jaundice for the study was 0.88 per cent. In treating more than 350 patients with low-dosage pyrazinamide over the last four years—most of them previously untreated patients in study groups but many whose organisms were resistant to the less toxic and better accepted antituberculous drugs—the incidence of jaundice has never exceeded 1 per cent and has usually been lower. It seems that only meticulous care in choosing patients for pyrazinamide therapy, together with careful liver screening every two weeks, has accomplished this. Others have received low-dosage pyrazinamide, yet have had higher jaundice rates. It has been argued by some that the available tests for liver involvement are not sensitive enough. The present writer does not agree with this. In each case in which jaundice has occurred in the present studies, the usual liver function tests have given ample warning. The important thing has been to stop the drug immediately when these tests change to any degree. For example, if the thymol turbidity which has been 2 units suddenly climbs to 8 units, the quantitative serum bilirubin which had been 0.2 mg. per 100 ml. climbs to 0.8 gm. per 100 ml., and the cephalin flocculation becomes 3- or 4-plus, pyrazinamide should be stopped at once. If the bromsulfalein excretion which has shown no retention should show 8 per cent or greater retention at forty-five

minutes, pyrazinamide should be stopped. The trouble appears to be that, when these tests are reported and are slightly elevated, pyrazinamide is sometimes continued, the liver function tests being repeated in a few days in the hope that the toxic elevations will have stabilized or become normal again. Such a gamble is very apt to result in jaundice during those few days when the drug is continued. One writer reported an incidence of jaundice of 3 per cent using a low dosage of pyrazinamide, but in 2 of his cases the patients were "moderately heavy alcohol drinkers."[4]

One of these patients was a woman. The only patient in the first study of the present writer (completed in 1956) who developed jaundice was the only woman in the study. Is it possible that pyrazinamide is more hepatotoxic in women, or is this merely the recognized phenomenon that drug-induced hepatitis may be more severe in women of the child-bearing age?

Recently Spengos and Cuizon[5] reported a death from acute yellow atrophy of the liver following treatment with low-dosage pyrazinamide. In this case, a pretreatment liver screening was done and was normal. A second screening was done two weeks later and was normal. Apparently the liver screenings were "not repeated frequently" after that "because the patient appeared to tolerate the drug well and no clinical evidence of liver embarrassment could be detected."

One cannot depend upon clinical signs and symptoms of early liver involvement. The liver function tests are imperative and should be done regularly at least every two weeks, but preferably weekly; certainly if any change toward the abnormal is noticed in the tests, these tests should be done weekly. Pyrazinamide is a dangerous hepatotoxic drug. Many other valuable drugs are also toxic in one way or another. It would appear to be established that pyrazinamide is a valuable asset to our armament against tubercle bacilli which also may kill the patient. It has been possible to bring many patients whose organisms were resistant to the more commonly accepted drugs to successful surgery by the use of pyrazinamide.

Patients are instructed to avoid alcohol while taking pyrazinamide or be faced with the likelihood of developing severe liver damage which may prove fatal. Pyrazinamide is stopped if a patient insists that he must leave the hospital for a few days on "pass." It is not always possible to be selective in the case of antituberculous drugs because many patients have infections which are resistant to isoniazid and streptomycin. To cure these patients with or without surgery, calculated risks have to be taken by using toxic drugs. If, however, very meticulous precautions are observed, it is believed that this risk will be rather low.

The rather low "substantial roentgenographic improvement" (moderate or marked) rate observed is due to the fact that this study, in contrast to the earlier study, was composed of many patients with minimal disease whose roentgenographic clearing could only be judged as "slight." For instance, if all degrees of roentgenographic improvement on *Regimen I* are considered, the improvement will be 90 per cent, whereas substantial improvement (moderate or marked) was only 50 per cent.

In summary: A group of 164 previously untreated patients were treated by three drug regimens. One hundred and thirteen of these patients received 1.5 gm. of pyrazinamide daily as one of the companion drugs. The over-all incidence of hepatic toxicity was 6.2 per cent as judged by liver function tests, whereas the incidence of jaundice was 0.88 per cent. There were no deaths from drug toxicity.

Pyrazinamide is a dangerous hepatotoxic drug which should be used only in patients whose tubercle bacilli are resistant to the less toxic drugs. Pyrazinamide is effective and may be life saving, so that the possible development of hepatitis constitutes a calculated risk, which is lessened to a considerable degree by frequent liver function tests.

STANTON T. ALLISON[6]
Veterans Administration Hospital
Rutland Heights, Massachusetts

June 23, 1958

[4] MUSCHENHEIM, C., *et al.*: Am. Rev. Tubere., 1955, *72*, 851.

[5] SPENGOS, T. N., AND CUIZON, R.: Am. Rev. Tubere., 1958, 77, 858.

[6] The writer wishes to acknowledge the great help of Mrs. Hilkka Hagman in so meticulously assembling the data in this study.

Correspondence

Method of Obtaining BCG

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

The recent report of the Ad Hoc Committee of the Surgeon General of the United States, after weighing the advantages and disadvantages of BCG vaccination, states: "It is believed, however, that the advantages of vaccination outweigh the disadvantages for tuberculin-negative persons who are exposed to a definite risk of infection, especially if they cannot be retested frequently with tuberculin."

This Committee recommended BCG vaccination for those groups recommended by the American Trudeau Society, the American College of Chest Physicians, the Council on Drugs of the American Medical Association, and the Medical Advisory Committee of Research Foundation of Chicago.

The specific groups recommended for BCG vaccination by the American Trudeau Society are:

1. Doctors, medical students, and nurses who are exposed to tuberculosis
2. All hospital and laboratory personnel whose work exposes them to contact with the bacillus of tuberculosis
3. Individuals who are unavoidably exposed to infectious tuberculosis in the home
4. Patients and employees in mental hospitals, prisons, and other custodial institutions in whom the incidence of tuberculosis is known to be high
5. Children and certain adults considered to have inferior resistance and living in communities in which the tuberculosis mortality rate is unusually high.

There have been numerous inquiries from all parts of the United States as to where BCG vaccine may be obtained. In order to assist those interested, the following information is offered:

BCG vaccine may be obtained by any licensed physician in the United States and its territories by writing to the laboratories of Research Foundation and the University of Illinois, the only institutions licensed to produce and distribute BCG in this country. The address of Research Foundation is: 70 West Hubbard Street, Chicago 10, Illinois.

Sol Roy Rosenthal
Research Foundation and
Institution for Tuberculosis Research,
University of Illinois
Chicago, Illinois

April 21, 1958

Treatment Failures

To the Editor of the American Review of Tuberculosis and Pulmonary Diseases:

During the 1958 annual meeting of the National Tuberculosis Association and the American Trudeau Society, there was a symposium on "Treatment Failures," defined by the panelists as a persistence of cavity and positive sputum after presumably adequate therapy had been afforded. When asked whether the patient's ability to return to work should be considered in determining whether treatment had failed or succeeded, the panelists agreed that return to work is a desideratum but not a necessity for evaluating therapy.

This attitude is reflected in most current literature on tuberculosis. More than twenty years ago, J. Arthur Myers pointed out that there are three objectives in treating the tuberculous person: to arrest the disease, prevent spread of infection, and restore social and economic usefulness. Can we talk intelligently of treatment success or failure without taking into account whether the patient is a better human being as a result of our endeavors?

Medical research has been devoted almost exclusively to tangible items susceptible of instrumental mensuration. There is a great inclination to attribute our failures to microbiologic resistance or inadequate therapy and to speak vaguely of "poor resistance" without really understanding what we mean by resistance. May it not be true that some of our thwarted efforts arise from failure to understand that resistance is only a name for a complex of physical, psychologic, emotional, and environmental factors unique to a given patient? In approaching the "hard core" of the tuberculosis problem perhaps we shall have to spend proportionately more time in social research to learn about those aspects of individual resistance which we can manipulate to the patient's advantage.

Sydney Jacobs

508 Medical Arts Building

New Orleans 15, Louisiana

October 1, 1958

Books

RESPIRATORY DISEASE AND THE GENERAL PRACTITIONER. By C. W. C. Toussaint. Pp. 28, National Association for the Prevention of Tuberculosis, London, England, 1958. Price, 7s.6d.

The new edition of this handbook on respiratory diseases by Dr. Hugh Toussaint has been enlarged to include chapters on the management of respiratory tuberculosis, the diagnosis of bronchogenic carcinoma, and a few pertinent remarks on bronchitis. In addition there are sections on normal chest roentgenographic interpretation, the pathogenesis of pulmonary tuberculosis, examination of the patient, and causes for delay in diagnosis. It is illustrated by 53 excellent roentgenographic reproductions. This reviewer is in hearty agreement with the forwarding remarks of Geoffrey Marshall that the booklet contains "a wealth of sound information and practical advice, with great economy of words." However, there are certain dogmatisms inevitable in a booklet of this size which are perhaps open to challenge, or which are perhaps applicable only to Great Britain. For example, the statement on page six that "The incidence of new cases (of tuberculosis) is highest among young workers" is certainly not true in this country where the highest incidence of new cases is being reported in elderly white males.

Dr. Toussaint believes that the correct positioning of the patient in bed is an important factor in cavity closure, a concept which has not been widely accepted in this country and, in addition, believes that all smoking must be forbidden. He stresses the gratifying results of chemotherapy if properly used and one infers from his remarks that the combination of all three major drugs is the drug regimen of choice in a fresh untreated case. One could argue with his statement that "the drugs can safely be stopped after one year." Dr. Toussaint makes many other points with which there can be no argument, such as the value of early diagnosis, the important role played by the general practitioner, and the unjustifiable excessive preoccupation with the risk of genetic damage being stirred up in the public mind in the case of chest roentgenography.

The section on bronchogenic carcinoma, which in England and Wales caused more than 18,000 deaths in 1956 compared with less than 5,000 in 1940, is clear and concise, and Dr. Toussaint believes that the only solution to this serious problem reaching almost epidemic proportions is prevention. He makes the important point that bronchogenic carcinoma may be present as an acute respiratory infection which may often respond to antimicrobials while the underlying cancer is unsuspected. No one can find fault with his statement that "it is wise to regard all abnormal chest shadows in persons over 40 years, as due to cancer until proved otherwise" or his insistence that there be the closest cooperation between general practitioner, radiologist, chest physician, thoracic surgeon, and pathologist in the clinical management of a case of bronchogenic carcinoma.

In the section on bronchitis there may be some disagreement with Dr. Toussaint's belief that antimicrobials are not essential in the treatment of the acute attack, and it is surprising that there is no mention of antimicrobials in the management of chronic bronchitis or the prophylactic role of these drugs in patients predisposed to repeated attacks of lower respiratory tract infections. Dr. Toussaint rightly condemns cigarette smoking for its important role in the etiology of chronic bronchial irritation.

Dr. Toussaint has included many concise and pertinent case histories in his text. Although directed primarily toward the general practitioner, this handbook should prove most valuable for the medical student and should be part of his recommended reading. It could also be read with considerable profit by the chest physician or internist with a particular interest in pulmonary diseases.

EDGAR ALSOP RILEY
New York, New York

CLINICAL ENZYMOLOGY. Edited by Gustav J. Martin. Pp. 241, Little Brown and Co., Boston, Massachusetts, 1958. Price, $6.00.

The archives of basic knowledge about enzymes are increasingly being put to use clinically. Under the editorship of Gustav J. Martin, the contributors have summarized some information of the present day pertaining to the diagnostic and therapeutic aspects of enzymology as they pertain to clinical situations. Although basic chemical discussion is included, this information is presented simply and only to the extent that a clinically oriented reader might find helpful and of interest. The bibliographic references are plentiful but not intended to be complete. The organization of the material is simple and clear, and subtitles within the seven chapters, as well as a good index, make for ready reference.

The therapeutic use of enzymes is well discussed; the diagnostic aspect of clinical enzymology is summarized, but lacks detail as well as clinical perspective. The chapter entitled "Polymerases in Biology," while interesting, does not seem to contribute appreciably to the main mission of the text. The first and last chapters are somewhat effusive, although the apparent justification as stated by the author was "to incorporate the field of clinical enzymology into a greater structure, into natural philosophy." Whether this purpose is worthy is uncertain; that it was not achieved is apparent. In a text on clinical enzymology some attention should have been given to the role of enzymatic defects in causation of disease, i.e., congenital galactosuria, phenylketonuria, hypophosphatasemia, hemolytic anemias, thrombin and blood clotting defects. This facet of clinical enzymology is not presented.

The book is an incomplete attempt to summarize clinical enzymology, and is recommended primarily to those who wish to be introduced to that aspect of enzymology concerned with the therapeutic use of enzymes. Increasing attention and interest in the expanding field of clinical enzymology demand a more ambitious and clinically oriented presentation.

FELIX WROBLEWSKI
New York, New York

SELECTED EXPERIMENTS IN MEDICAL MICROBIOLOGY. By Stewart M. Brooks. Pp. 79, W. B. Saunders Company, Philadelphia, Pennsylvania, 1958. Price, $2.00.

This is a companion to the author's book *Basic Facts of Medical Microbiology*. It is in essence a simple manual of instruction in a few microbiologic procedures, from the use of the microscope to the recognition of microorganisms. There is in addition a not-so-simple questionnaire between each "experiment."

The author does not claim that this book represents a comprehensive course in practical microbiology, but it should prove useful in any introductory course in the practical aspects of bacteriology.

JOHN BATTEN
London, England

BOOKS RECEIVED

THE MANAGEMENT OF EMERGENCIES IN THORACIC SURGERY. By John Borrie. Pp. 340, Appleton-Century-Crofts, Inc., New York, 1958. Price $10.00.

DOUZIÈME CONGRÈS NATIONAL DE LA TUBERCULOSE. Published with the aid of Professeur Bariety. Pp. 204, Masson et Cie, Editeurs, Paris, 1958. Price, 2500 fr.

LES HÉMOPTYSIES TRACHÉO-BRONCHIQUES. By André Meyer and Jacques Chrétien. Pp. 158, Masson et Cie, Editeurs, Paris, 1958. Price, 2,000 fr.

PHYSIOTHERAPY IN CHEST DISEASES. By Torsten Bruce, Caroline Reuterswärd, and Birgit Westin. Pp. 98, National Association for the Prevention of Tuberculosis, London, 1958. Price, 12s.6d.

AN ATLAS OF ESOPHAGEAL MOTILITY IN HEALTH AND DISEASE. By Charles F. Code *et al.* Pp. 134, Charles C Thomas, Publisher, Springfield, Illinois, 1958. Price, $8.50.

EPIDEMIOLOGIA GRŮZLICY. By Marian Zierski. Pp. 163, Państwowy Zakład Wydawnictw Lekarskich, Warsaw, 1958.

DIE ENTWICKLUNG DER TUBERKULOSEBEHANDLUNG SEIT 100 JAHREN. Edited by G. Domagk, L. Heilmeyer, and H. Krauss. Pp. 47, Georg Thieme Verlag, Stuttgart, 1958. Price, $2.85.

NEGROES AND MEDICINE. By Dietrich C. Reitzes. Pp. 400, Harvard University Press, Cambridge, Massachusetts, 1958. Price, $7.00.

HANDBOOK OF RESPIRATION. Analysis and Compilation by Philip L. Altman, John F. Gibson, Jr., and Charles C. Wang. Pp. 403, W. B. Saunders Company, Philadelphia, 1958. Price, $7.50.

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Raymond C. McKay, M.D., *Chairman*
Cuyahoga County Hospital
3395 Scranton Road
Cleveland 9, Ohio

James D. Murphy, M.D.
3900 Loch Raven Boulevard
Baltimore 18, Maryland

Edward Kupka, M.D.
2151 Berkeley Way
Berkeley 4, California

Frederick C. Warring, Jr., M.D.
Laurel Heights Sanatorium
Shelton, Connecticut

Committee on Foreign Affairs

Theodore L. Badger, M.D., *Chairman*
264 Beacon Street
Boston 16, Massachusetts

Howard T. Barkley, M.D.
4414 Montrose Boulevard
Houston 6, Texas

John D. Steele, M.D.
Veterans Administration Hospital
San Fernando, California

William Steenken, Jr., D.Sc.
Trudeau Laboratory
Saranac Lake, New York

Committee on Program Activities

David T. Carr, M.D., *Chairman*
Mayo Clinic
Rochester, Minnesota

Theodore L. Badger, M.D.
264 Beacon Street
Boston 16, Massachusetts

Katharine R. Boucot, M.D.
Woman's Medical College
Philadelphia 29, Pennsylvania

Robert H. Browning, M.D.
Ohio Tuberculosis Hospital
Columbus 10, Ohio

Martin M. Cummings, M.D.
Veterans Administration
Research Division
Washington 25, D. C.

Michael L. Furcolow, M.D.
University of Kansas Medical Center
Kansas City 12, Kansas

H. William Harris, M.D.
4599 Highland Drive
Salt Lake City, Utah

Kirby S. Howlett, Jr., M.D.
Laurel Heights Sanatorium
Shelton, Connecticut

Raymond C. McKay, M.D.
Cuyahoga County Hospital
3395 Scranton Road
Cleveland 9, Ohio

William H. Oatway, Jr., M.D.
La Vina Sanatorium
Altadena, California

H. McLeod Riggins, M.D.
140 East 54 Street
New York 22, N. Y.

Margaret H. D. Smith, M.D.
New York University College of Medicine
550 First Avenue
New York 16, New York

Joseph B. Stocklen, M.D.
East Side County Clinic
4520 Carnegie Avenue
Cleveland 3, Ohio

William B. Tucker, M.D.
Veterans Administration
Tuberculosis Service
Washington 25, D. C.

Col. James A. Wier, M.C.
Fitzsimons Army Hospital, Box 308
Denver 8, Colorado

Robert L. Yeager, M.D.
Summit Park Sanatorium
Pomona, New York

Committee on Radiation Effects

Joseph B. Stocklen, M.D., *Chairman*
East Side County Clinic
4520 Carnegie Avenue
Cleveland 3, Ohio

E. Osborne Coates, Jr., M.D.
Henry Ford Hospital
Detroit 2, Michigan

Richard H. Chamberlain, M.D.
3400 Spruce Street
Philadelphia 4, Pennsylvania

John H. McClement, M.D.
Bellevue Hospital—Chest Service
462 First Avenue
New York 16, New York

Clinton Powell, M.D.
Public Health Service
National Institutes of Health
Bethesda 14, Maryland

Committee on Diagnostic Skin Testing

Michael L. Furcolow, M.D., *Chairman*
University of Kansas Medical Center
Kansas City 12, Kansas

Cabot Brown, M.D.
450 Sutter Street
San Francisco 8, California

Helen A. Dickie, M.D.
1300 Wisconsin Avenue
Madison 6, Wisconsin

Lydia B. Edwards, M.D.
Division of Special Health Services
Public Health Service
Washington 25, D. C.

Florence B. Seibert, Ph.D.
Henry Phipps Institute
Seventh and Lombard Streets
Philadelphia 47, Pennsylvania

David T. Smith, M.D.
Duke Hospital
Durham, North Carolina

Committee on Tuberculosis in Children

Margaret H. D. Smith, M.D., *Chairman*
New York University College of Medicine
550 First Avenue
New York 16, New York

Miriam E. Brailey, M.D.
4422 Marble Hall Road
Baltimore 18, Maryland

John S. Chapman, M.D.
5323 Harry Hines Boulevard
Dallas 19, Texas

Katharine H. K. Hsu, M.D.
2022 Rosedale Street
Houston 4, Texas

Edna M. Jones, M.D.
William H. Maybury Sanatorium
Northville, Michigan

Edwin L. Kendig, Jr., M.D.
3603 Grove Avenue
Richmond 21, Virginia

Committee on Roster

Robert L. Yeager, M.D., *Chairman*
Summit Park Sanatorium
Pomona, New York

Howard W. Bosworth, M.D.
1301 Chavez Ravine Road
Los Angeles 26, California

Ralph E. Dwork, M.D.
492 S. Drexel Avenue
Columbus 9, Ohio

George J. Wherrett, M.D.
265 Elgin Street
Ottawa, Ontario, Canada

Committee on Diagnostic Standards and Classification of Tuberculosis

William H. Oatway, Jr., M.D., *Chairman*
La Vina Sanatorium
Altadena, California

David Salkin, M.D.
Veterans Administration Hospital
San Fernando, California

Peter A. Theodos, M.D.
1930 Chestnut Street
Philadelphia 3, Pennsylvania

Norman J. Wilson, M.D.
135 Francis Street
Boston 15, Massachusetts

Emanuel Wolinsky, M.D.
Cuyahoga County Hospital
3395 Scranton Road
Cleveland 9, Ohio

ATS Representatives on National Tuberculosis Association Committees

Budget Committee
Daniel E. Jenkins, M.D.
3550 Sun Valley Drive
Houston 25, Texas

Roger S. Mitchell, M.D.
4200 East Ninth Avenue
Denver 20, Colorado

Committee on Cooperation with Federal Agencies
Robert L. Yeager, M.D.
Summit Park Sanatorium
Pomona, New York

Elliot Mendenhall, M.D.
Medical Arts Building
Dallas, Texas

Committee on TB Among American Indians
H. Crawford Jernigan, M.D.
Fort Stanton TB Hospital
Fort Stanton, New Mexico

Committee on International Affairs
Daniel E. Jenkins, M.D.
3550 Sun Valley Drive
Houston 25, Texas

Alternate
Roger S. Mitchell, M.D.
4200 East Ninth Avenue
Denver 20, Colorado

Committee on Nursing
Michael L. Furcolow, M.D.
University of Kansas Medical Center
Kansas City 12, Kansas

Committee on Policy
Theodore L. Badger, M.D.
264 Beacon Street
Boston 16, Massachusetts

Ross Medal Committee
J. Burns Amberson, M.D.
New York TB and Health Association
260 Fourth Avenue
New York 10, New York

Committee on Social Research
Raymond C. McKay, M.D.
Cuyahoga County Hospital
3395 Scranton Road
Cleveland 9, Ohio

Trudeau Medal Committee
William B. Tucker, M.D.
Tuberculosis Service
Veterans Administration
Washington 25, D. C.

Committee on Christmas Seal Sale
Howard T. Barkley, M.D.
4414 Montrose Boulevard
Houston 6, Texas

Committee on Respiratory Diseases
David T. Carr, M.D.
Mayo Clinic
Rochester, Minnesota

Roger S. Mitchell, M.D.
4200 East Ninth Avenue
Denver 20, Colorado

Obituaries

Samuel S. Cooley

1905–1958

Dr. Samuel S. Cooley died in Asheville, North Carolina, on May 2, 1958, as a result of coronary thrombosis. Dr. Cooley was born in 1905 in Trenton, New Jersey. He obtained his medical degree from the New York University College of Medicine in 1934, and did postgraduate work at Bellevue Hospital and at the University of Pennsylvania Graduate School of Medicine.

Dr. Cooley was a Diplomate of the American Board of Internal Medicine. He served as a captain in the Medical Corps of the United States Army from 1942 to 1945. He was a member of the staff of the Memorial Mission Hospital in Asheville and a consultant to the Western North Carolina Sanatorium in Black Mountain and the Veterans Administration Hospital at Oteen, North Carolina. Dr. Cooley's humanitarianism was appreciated by his patients, and his clinical perception was respected by his colleagues.

J. D. M.

Frederick James Gray, Jr.

1911–1958

Dr. Frederick J. Gray, Jr., of Little Rock, Arkansas, died of a coronary occlusion in Hot Springs, Arkansas, on May 7, 1958. Dr. Gray graduated in medicine at Vanderbilt University in 1943. He took graduate work in surgery at Columbia, Missouri, Nashville, Tennessee, and at the Veterans Hospital, Oteen, North Carolina, where he specialized in thoracic surgery.

Dr. Gray was certified by the American Board of Surgery and the Board of Thoracic Surgery. He was an assistant clinical professor of surgery in the University of Arkansas Medical School and a consultant to the Little Rock Veterans Administration Hospital. He was a veteran of World War II.

Dr. Gray was a Fellow of the American College of Surgeons, a member of the Southern Thoracic Surgical Association, the American Trudeau Society, and of the county, state, and American Medical associations. He was a former President of the Arkansas Trudeau Society.

Dr. Gray was dedicated to the practice of thoracic surgery. During his years of resident training his surgical skills, his devotion to medicine, and his sympathetic relationships with the patients under his care gave evidence of the contributions he would later make in the field of surgery and pulmonary disease.

In addition to his medical activities he participated in religious, civic, and community affairs in his area where he was active in the Little Rock Rotary Club, the Westover Hills Presbyterian Church, and in Boy Scout work.

He is survived by his widow, Lavona Gray, two sons, and a daughter.

J. D. M.

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1959—Chicago, Illinois—May 24–29

1960—Los Angeles, California—May 15–20

ERRATUM

In the Note entitled "A Semi-Synthetic Autoclavable Medium for a Routine Tuberculosis Laboratory," by Lenore R. Peizer *et al.*, in the November, 1958, issue of the REVIEW (vol. 78, p. 788), the first item of the formula on p. 789 should have read "Sodium glutamate ($C_5H_8NNaOH_4$) . . . *4.0 gm.*